HYBRIDS OF *OENOTHERA BIENNIS* AND *OENOTHERA FRANCISCANA* IN THE FIRST AND SECOND GENERATIONS[1]

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INTRODUCTION

This paper will give the results of a study involving two species of Oenothera and their hybrids in the first and second generations. The species, *Oenothera biennis* Linnaeus and *Oe. Franciscana* Bartlett, are sharply contrasted in a color character as well as in characters of form and size, and consequently offer favorable material to test the problem of whether or not a process of protoplasmic differentiation at the end of the F_1 generation leads to the formation of diverse types of gametes, making possible a segregation of characters and the consequent establishment of distinct classes in the F_2 generation. Such a behavior, if clearly evident, would naturally point towards a Mendelian interpretation of the results, if it be admitted that the chief tenet of Mendelism is the principle of segregation following an F_1 generation.

[1] Genetical studies on Oenothera—VII.

The observations on certain of the cultures in the F_2 have an especial interest in being, so far as I know, the first cultures of Oenothera grown, in which the germination of seeds has been experimentally forced to a finish, giving cultures which at the beginning contained the progeny of all viable seeds. Thus a method is introduced in genetical work among oenotheras which the writer believes to be absolutely essential to accurate and trustworthy results in this group of plants. The evidence is clear that large proportions of Oenothera seeds sprout in the earth only after many weeks or even months and that this habit of delayed germination must have given in many of the cultures described in the Oenothera literature hardly more than glimpses of the genetical possibilities. We cannot feel certain that the records of any cultures of Oenothera so far reported are complete for their possible progeny, and consequently the ratios of classes described in breeding experiments and the percentages of "mutants" calculated cannot be accepted as final in exact genetical work. We are not in a position even to guess what may be the changes of front when exact data become available.

The views of DE VRIES have been in general strongly against Mendelian interpretations of his results with the exception of the behavior of *Oenothera brevistylis* which appears to act as a recessive in crosses with *Lamarckiana* and other species. Recently, however, DE VRIES (1915 c) reports the isolation of certain forms of *Oe. gigas* which, when selfed, gave about 18 percent dwarfs, about 25 percent pure *gigas,* and about 57 percent of *gigas*-like "mutant hybrids" similar to the parent plants and which, when selfed, again threw about 21 percent of dwarfs. These impure *gigas* forms are regarded by DE VRIES as being heterozygous for a dwarf factor and consequently exhibiting in their breeding the simple Mendelian ratio of a monohybrid.

Other investigators have in recent years with varying emphasis expressed their doubts as to the applicability of Mendelian principles to various features of Oenothera genetics. Thus GATES (1914, 1915 a), largely on studies of crosses between *Oenothera rubricalyx* and *Oe. grandiflora,* came strongly to the conclusion that the "mutants" of Oenothera cannot be explained as the appearance of types thrown out by the Mendelian behavior of hybrid material. A recent paper (GATES 1915 b), nevertheless, argues for Mendelian interpretations of certain results. In all of the work of GATES we can have no confidence that his data truly express the genetical situations since the cultures were frequently small and all were apparently grown without consideration of the probability of extensive delayed germination and seed-sterility, and

cannot be expected to give an accurate record of the conditions. SHULL (1914) in a paper on *rubricalyx* in crosses with *Lamarckiana* and *rubrinervis* announces his belief that "a hereditary mechanism must exist in Oenothera fundamentally different from that which distributes the Mendelian unit-characters." SHULL's cultures also were grown from seed sown in the earth and are without data on delayed germination and seed-sterility; they also are likely imperfectly to represent the true genetical situation. The work with *rubricalyx* is at present much clouded by the discussion between GATES and SHULL over the origin and genetical constitution of this remarkable plant. BARTLETT (1915 a, b, c, d) in a series of papers has given us the most impressive evidence in recent years for mutation in Oenothera, together with arguments against its interpretation as Mendelian phenomena, but the writer (DAVIS 1915 b, c) has already expressed his belief that such studies can only have full value when the material is most thoroughly tested for its genetical purity and when germinations in the cultures are proved to be complete. There is evidence of order in BARTLETT's results, to me in the main very suggestive of Mendelian behavior.

In a paper on hybrids between an American *"Oenothera biennis"* and *Oe. grandiflora* the writer (DAVIS 1912, 1913 a) pointed out what then seemed to him difficulties in interpreting conditions exhibited by the F_2 in accordance with a strict Mendelian conception of the stability and conservation of factors. It was a tentative position of which EAST (1912) has presented pertinent criticism and to which I now attach no importance since there is every reason to believe that my methods of culture at that time, in common with those of all other workers on oenotheras, did not give exact genetical data because the seed-germination was undoubtedly incomplete and also for the reason that the parent stocks had not passed sufficiently critical tests for their genetic purity. I have recently discussed the tests that must be applied to a species of Oenothera to determine whether or not it is genetically pure (DAVIS 1915 b) and will only emphasize my belief that no type has as yet satisfied such an examination. Consequently we have at present in the oenotheras no standard material of genetic purity with which forms under suspicion may be confidently mated to determine by cross-breeding the uniformity of their viable gametes. Until such material is discovered we shall be working largely in the dark in our attempts to analyse the genotypic constitution of oenotheras.

Two writers on Oenothera genetics, HERIBERT-NILSSON and RENNER, have taken a clear Mendelian attitude in the interpretation of their re-

sults. HERIBERT-NILSSON (1912, 1915) in lengthy papers reports studies on a race of *Lamarckiana* from Sweden. He holds the view, with which I am in complete sympathy, that *Lamarckiana* is an impure or polymorphic species, of hybrid constitution. From this standpoint he develops the theory that its "mutants", which he lists as "combinations", are the result of various assemblings of Mendelian factors following the processes of segregation during the reduction divisions in the parent forms. It is a promising line of study which HERIBERT-NILSSON has followed with persistence. However, because of his methods of sowing seeds in the earth without proper check on the probabilities of incomplete germination we cannot be certain that he has obtained full results in the data which he has gathered, either as to the forms themselves or as to their ratios in the cultures.

We are indebted to RENNER (1914) for the first critical consideration of seed-sterility in Oenothera and its possible bearing on the genetical constitutions of forms whose breeding habits are peculiar. He is the first investigator to suggest that seed-sterility in this group may mask a behavior essentially Mendelian in its character. This attitude is well illustrated by his suggestive hypothesis on the genotype of *Lamarckiana*. Since *Lamarckiana* when crossed with *biennis* and certain other species gives the twin hybrids *laeta* and *velutina* it may be assumed to develop two types of gametes which function, and these may be called the *laeta* and *velutina* types. *Lamarckiana* self-pollinated might then give zygotes in the proportions of 1 pure *laeta: 2 laeta-velutina:* 1 pure *velutina*. Now more than one half of the seeds of *Lamarckiana* fail to develop normal embryos and RENNER believes that in these sterile seeds are represented zygotes homozygous respectively for the *laeta* and *velutina* factors. The fertile seeds are believed by RENNER to develop from the heterozygotes with *laeta* and *velutina* factors acting together and this combination is held to give the characters of *Lamarckiana*. *Oenothera Lamarckiana* is thus considered by RENNER to be an impure or heterozygous species, breeding true because of the mortality of such zygotes as carry the factors for *laeta* and *velutina* in homozygous conditions. The hypothesis offers a simple Mendelian explanation of the behavior of *Lamarckiana* in producing twin hybrids, and the facts of seed-sterility support the view of its genetic impurity.

The studies of RENNER on seed-sterility are certain to have a profound effect on future investigations in Oenothera genetics. DE VRIES (1915 a, b) recognized their importance at once by working out an experimental method by which rapid germination may be obtained through forcing

water into wet seeds by increased air pressure, thus readily obtaining the percentages of germination. Last winter I also took up the problem and tested the seed-fertility of some fifty species, races or hybrids, and I have already published the results of the study for a number of hybrids between *biennis* and *muricata* (DAVIS 1915 b). The immediate result of this understanding of the importance of seed-sterility and delayed germination is likely to be the adoption in the future of methods whereby the germination of seeds will be brought to completion and the residue of sterile seed-like structures preserved for examination.

METHODS OF OBTAINING COMPLETE GERMINATION OF SEEDS

Certain F_2 generations reported in this paper were grown in the past summer (1915), following an experimental method that gave a complete germination of the viable seeds, as proved by the examination of the sterile seed-like structures.

The method has been described in full (DAVIS 1915 a) and will be only briefly outlined at this time. The seeds were germinated on pads of wet filter paper in Petri dishes kept in a warm portion of a hot house. The sprouted seeds with radicles 3 mm or more in length were then set in sterilized soil in square seed-pans and were arranged and properly spaced in definite rows. Thus the seedlings were counted as planted and the death of any became evident. The young rosettes were later transferred from the seed-pans to small pots where they generally became ¼ to ⅓ full grown before they were set out in the garden. The residue of sterile seed-like structures left in the Petri dish was then examined, after germination appeared to be complete, to make sure that no viable seeds remained. The examination is easily made by pressing the structures, now softened by water, between the tips of a strong pair of forceps; any embryos present are at once squeezed out and may be readily examined. The sterile seeds were conveniently arranged, as illustrated in figure 13, and then fastened to the piece of filter paper by flooding the seeds with a solution of shellac in alcohol, thus preserving a record of the residue.

This method is, I think, open to an important improvement which will be tested in future cultures. Following the suggestion of DE VRIES (1915 a, b) I shall subject the thoroughly wet seeds to increased air pressure before placing them in the Petri dishes. This may be readily accomplished by placing test tubes, containing the seeds immersed in water, within a metal conceptacle that may be closed air-tight and into which air may be forced by an automobile pump to a pressure of several atmo-

spheres indicated by a gauge. By thus forcing water into the seeds DE VRIES claims that germination will take place immediately and there should not be a long wait in the Petri dishes before germination is complete.

This paper will also describe certain cultures grown in 1914 after the old methods of sowing seeds in the earth. Following my practice of recent years I counted the seed-like structures that entered the seed-pans and in this way obtained a fair idea of the proportions of seeds which germinated after being in the pans for 8-10 weeks. But it has become evident that delayed germinations under such conditions make impossible accurate genetical work since seeds sown in earth are obviously lost for

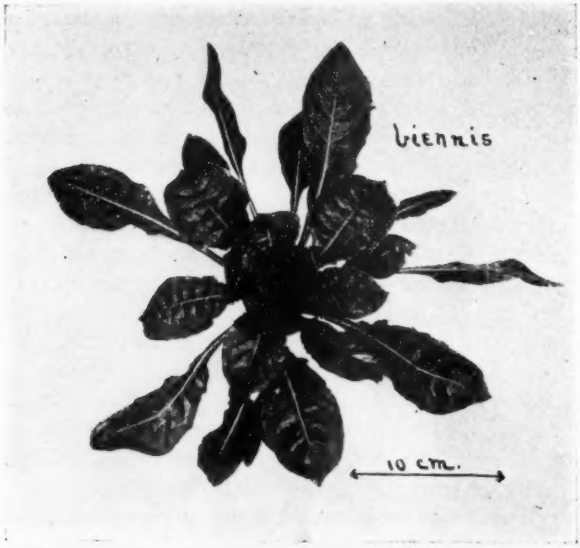

FIGURE 1.—Rosette of *Oenothera biennis*, nearly mature, showing the close cluster of broadly-elliptical, crinkled leaves. Contrast with figure 2, *Franciscana*.

further inquiry as to the fact of their viability; a proportion of seedlings appear, but, as for the residue, that cannot be examined.

I have laid emphasis on the necessity of developing methods of experimental germination of seeds in genetical work in Oenothera for reasons outlined in the introduction. The practice of sowing seeds directly in the earth must always be open to sharp criticism because of the conditions of seed-sterility and the habit of delayed germination prevalent in this group

of plants. It may again be noted that none of the published research in Oenothera genetics has as yet satisfied the conditions of the problems and that none of the results so far as they report ratios of classes, segregates, or "mutants," can be regarded as fully trustworthy.

A COMPARISON OF THE PARENT SPECIES, *OENOTHERA BIENNIS* LINNAEUS AND *OE. FRANCISCANA* BARTLETT

The race of *Oenothera biennis* employed was that described in my paper "Some reciprocal crosses of Oenothera" (DAVIS 1914). It is the plant made familiar to us by the studies of DE VRIES and STOMPS and

FIGURE 2.—Rosette of *Oenothera Franciscana* B, nearly mature, showing the close cluster of narrower leaves, less strongly crinkled. Contrast with figure 1, *biennis*.

represents a type wide-spread on the sand dunes of Holland and also present in England and in other European countries. BARTLETT (1913) has given strong reasons why it should be regarded as the form known to Linnaeus as *Oenothera biennis* and consequently to be regarded as the type form of the species. This Dutch plant in garden cultivation has proved to be remarkably stable. However, STOMPS (1914) in cultures

of selfed lines from a single plant collected in 1905, discovered that the Dutch *biennis* throws occasional marked variants ("mutants"). De Vries (1915 a) in large cultures totaling 8500 plants from the same lines established by Stomps, found that the three variants appeared in the following percentages, (1) *biennis semi-gigas* (21 chromosomes and self-sterile) about 0.05 percent, (2) *biennis nanella* (dwarf) about 0.1 percent, and (3) *biennis sulfurea* (a variety with pale yellow petals) about 0.3 percent. Since the percentages from *Lamarckiana* are for *semi-gigas* 0.3 percent and for *nanella* 1 to 2 percent, it may be noted that with respect to these "mutants" *biennis* appears to be the more stable of the two species. The color variety *biennis sulfurea* is a type of variant new in experimental studies on oenotheras and it is interesting to record that a single plant exhibiting its peculiarity appeared in the F_2 of a cross *biennis* × *Franciscana* B described on page 243 of this paper.

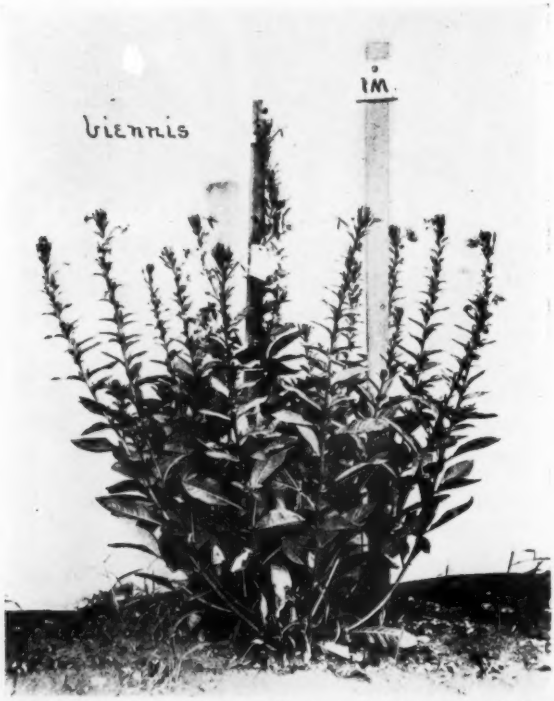

Figure 3.—Mature plant of *Oenothera biennis*. Showing long side-branches and a foliage of large, broadly-elliptical leaves. Contrast with figure 4, *Franciscana*.

The species *Oenothera Franciscana* was recently isolated by BARTLETT (1914) from among the oenotheras of the Pacific coast. I have this species now from two sources: (1) *Franciscana* B, the strain which BARTLETT obtained from seed collected at Carmel Beach, Monterey, California, and (2) *Franciscana* E from seed collected by Miss EASTWOOD in San Mateo County, California. Both races have been uniform through three seasons of garden culture of 52, 18 and 16 plants for *Franciscana* B and 140, 18 and 16 plants of *Franciscana* E. Although at present we know the species only from the region of central California, where it appears to be not uncommon, there seems to be no reason why it may not prove to be widely distributed in the western and southwestern United States. I saw last summer in the garden of Professor BARTLETT cultures of two oenotheras clearly in the *Franciscana* alliance, one from the Santa Rita mountains, and the other from the Santa Catalina mountains, in Arizona.

FIGURE 4.—Mature plant of *Oenothera Franciscana* B. Showing shorter side-branches and a foliage of smaller, narrower leaves. Contrast with figure 3, *biennis*.

There are no essential taxonomic differences between these two races of *Franciscana* from California except that *Franciscana* E appears to be somewhat more vigorous. Nevertheless, there have been important differences between the F_2 progenies in crosses with *biennis,* and *Franciscana* B has given a more varied assortment of forms than *Franciscana* E in spite of the fact that the F_2 cultures of the crosses with *Franciscana* E were from seeds in which germination was experimentally established to be complete.

My first crosses were made between *biennis* and *Franciscana* B with the assistance of BARTLETT who had called my attention to the characters of *Franciscana* as likely in combination with those of *biennis* to give hybrids resembling the *Lamarckiana* of DE VRIES. We made in 1912 reciprocal crosses between a plant of *Franciscana* B grown in Washington and a plant of *biennis* in Philadelphia, exchanging pollen by post, and the hybrids from these two crosses furnished the material for part of this paper. Since then I have made reciprocal crosses between *biennis* and *Franciscana* E and carried hybrids through the second generation.

To bring the contrasting characters of *Oenothera biennis* and *Oe. Franciscana* clearly before the reader the two species will first be described in parallel columns. The descriptions are of plants started under glass during the winter and in the spring set out as rosettes in the experimental garden. By this treatment the biennial habit may be broken and the plants brought to maturity during the summer.

Oenothera biennis Linnaeus	*Oenothera Franciscana* Bartlett
Cotyledons. 6—7 mm long, broad at the base and short-petioled.	Cotyledons. 8—9 mm long, broadly elliptical and long-petioled.
Young rosettes. A close cluster of broadly-elliptical or ovate leaves, short-petioled.	Young rosettes. A close cluster of elliptical leaves, narrower and long-petioled.
Mature rosettes. 3.5—4 dm broad. A close cluster of broadly-elliptical leaves tapering to relatively short petioles, crinkled (figure 1), mid-veins reddish. Central shoot generally appearing from the rosette before the side branches.	Mature rosettes. 3.5—4.5 dm broad. A close cluster of narrower leaves, somewhat spatulate and longer petioled, less strongly crinkled (figure 2), mid-veins reddish. Side branches from the rosette frequently appearing before the central shoot.

Mature plants. About 1 m high. Numerous long side-branches from the rosette, in length almost equal to the central shoot (figure 3). Papillae at the base of long hairs follow the color of the clear green stems. Green stems with a scant pilose pubescence.

Foliage. Leaves broadly elliptical, a lighter green, thin, large (figures 3 and 5).

Inflorescence. Compact. Bracts ¼—½ length of mature buds, broad near the base (figure 5).

Mature buds. 5.5—6 cm long. Cone 2—2.3 cm long, slightly 4-angled. Sepals green, less heavily pilose and puberulent. Sepal tips 3—4 mm long, slender. Papillae on ovaries green.

Petals. 2—2.3 cm long, a golden yellow.

Stigma lobes. 4—5 mm long, low, generally about 3 mm below the tips of the anthers. Close pollinated.

Capsules. 2—2.3 cm long, stout, pilose and puberulent.

Pollen. About 50 percent abortive.

Seeds. Almost wholly fertile.

Mature plants. 1—1.2 m high. Numerous long side-branches from the rosette, shorter than the central shoot (figure 4). Papillae at the base of long hairs are bright red over the green stems. A heavy pilose pubescence from the red papillae.

Foliage. Leaves narrow elliptical, a darker green, thicker, relatively small (figures 4 and 6).

Infloresence. More open. Bracts ⅓—½ length of mature buds, narrower (figure 6).

Mature buds. 8.5—9 cm long. Cone 3.2—3.5 cm long, strongly 4-angled. Sepals streaked with red, heavily pilose and puberulent. Sepal tips 5—6 mm long, thick. Papillae on ovaries red.

Petals. 3—3.5 cm long, a lighter yellow.

Stigma lobes. 4—5 mm long, high, from 5—7 mm above the tips of the anthers. Open pollinated.

Capsules. 2.8—3 cm long, slender, heavily pilose and puberulent.

Pollen. Few abortive grains.

Seeds. About 60 percent fertile.

From the above descriptions and by comparison of the figures it may readily be seen that the two species differ conspicuously in many points of size, form and relation of their parts. The chief contrasting features of morphology concern (1) the form of the rosette leaves (figures 1 and 2), (2) the foliage of the mature plants (figures 3 and 4, 5 and 6), (3) pubescence of the stems, (4) the form of the bracts (figures 5 and 6), (5) the size of the buds, form of the sepal tips and relative density of the pubescence on the sepals, (6) the size of the petals, (7) the relative positions of the stigma lobes with reference to the tips of the anthers, and (8) the form and length of the capsules and density of their pubescence.

With respect to characters of color there is (1) a difference in the shade of yellow in the petals, (2) the presence of red streaks on the sepals of *Franciscana* and a greater amount of red over old portions of the stems, and (3) in *Franciscana* the bright red coloration of papillae over the green stems and ovaries; sepals and papillae in *biennis* are green. Differences in the depth of yellow in the petals proved very difficult to distinguish and I was unable to follow such differences accurately

FIGURE 5.—Inflorescence of *Oenothera biennis*. Showing its compact structure and broad bracts. At the left is a leaf from near the base of the main stem. Contrast with figure 6, *Franciscana*.

through the hybrids. The depth of red in the sepals of the hybrids likewise presented so many degrees of intensity, obviously related to the age of the plant, that it also proved unsatisfactory for a genetical

study. The color of the red papillae on stems and ovaries was, however, always brilliant, so that I never had doubts of its presence in my classification of hybrid material.

Since I have given especial attention to the behavior of red papillae in hybrids of the F_1 and F_2 generations it is important to examine the evidence of the purity of the parents with respect to the presence of this character in *Franciscana* and its absence in *biennis*. DE VRIES and

FIGURE 6.—Infloresence of *Oenothera Franciscana* B, showing its more open structure and narrower bracts. At the right is a leaf from near the base of the main stem. Contrast with figure 5, *biennis*.

STOMPS in letters to me report that they have never noted red papillae on the stems of *biennis* in their cultures of many thousands of individuals and my cultures derived from seed of DE VRIES have for the past four

years been uniformly green-stemmed. We know less of *Franciscana*, but BARTLETT and I have between us grown several hundred plants and red papillae have always appeared in full and striking coloration.

It will be of interest to think for a moment of these two species, *biennis* and *Franciscana*, with reference to the characters that each may contribute to the synthesis of hybrid types resembling the *Lamarckiana* of DE VRIES's cultures. The mature rosettes of *Lamarckiana* present leaves in form somewhat between those of *biennis* and *Franciscana* but more crinkled. *Lamarckiana* has the same habit of sending up long side-

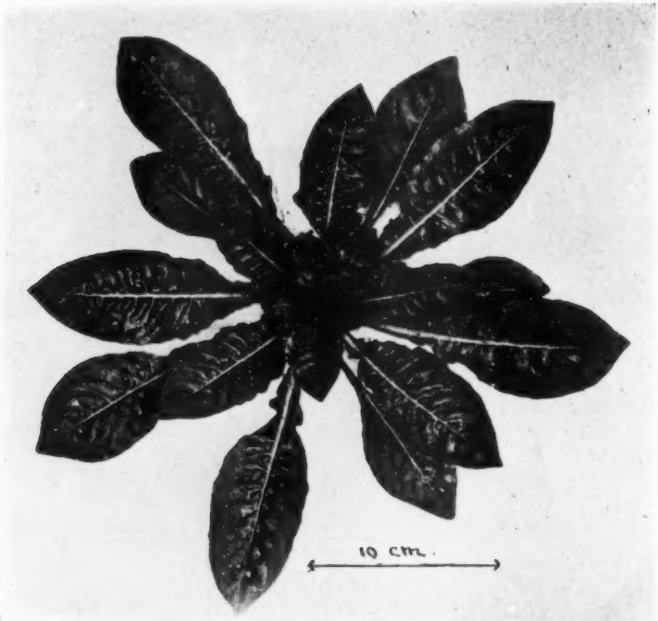

FIGURE 7.—Rosette of the F_1 hybrid, 14.37 *ac*, *Franciscana* E × *biennis*. Clear evidence of a blend in the form of the leaves with, however, patroclinous tendencies. Compare with figure 1, *biennis*, and contrast with figure 8, *biennis* × *Franciscana* E.

branches from the rosettes, its leaves are large as in *biennis* although more crinkled, its green stems bear red papillae at the base of long hairs as in *Franciscana*. The inflorescence of *Lamarckiana* combines large buds and large flowers as in *Franciscana* with bracts more like those of *biennis*. Its buds are about the same size as those of *Franciscana* but the cone is somewhat stouter and not so strongly 4-angled; the sepals

are sometimes faintly streaked with red, conspicuously so in *rubrinervis* which has very much the color of *Franciscana;* the sepal tips in form are more like those of *biennis* but longer; the pubescence of the sepals is intermediate between these two species; the ovaries have red papillae as in *Franciscana.* The flowers of some forms of *Lamarckiana* are somewhat larger (petals 4—4.5 cm) than those of *Franciscana,* in other forms (petals 2.5—3 cm) smaller; the petals are a golden yellow as in *biennis;* the position of the stigma lobes in the large-flowered forms, 5—7 cm above the tips of the anthers, is as in *Franciscana.* The cap-

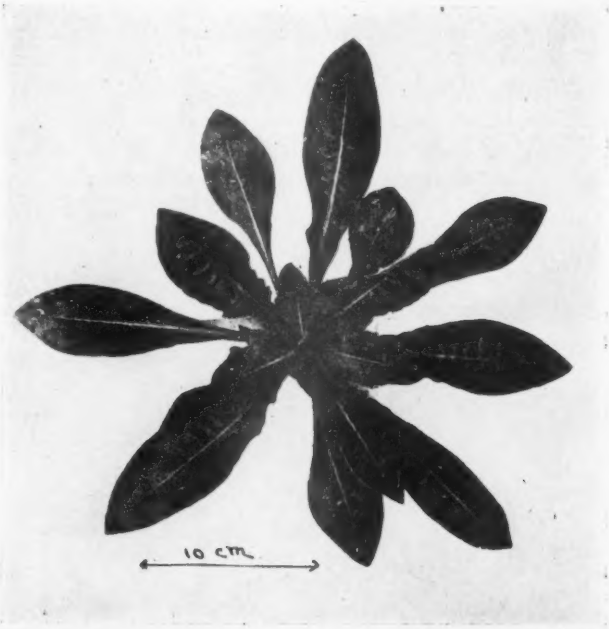

FIGURE 8.—Rosette of F_1 hybrid, 14.38 *a*, *biennis* × *Franciscana* E. More strongly patroclinous in the form of the leaves and the lesser degree of their crinkling. Compare with figure 2, *Franciscana,* and contrast with figure 7, *Franciscana E* × *biennis.*

sules of *Lamarckiana* are similar to those of *biennis* in form and size although more variable, exhibiting a considerable range in different races. Thus we see that *biennis* and *Franciscana* have between them characters which, segregating in pure or somewhat modified forms in the F_2 or later generations, might in some individuals readily present combinations that would give *Lamarckiana*-like types. I believe that this is exactly what

has happened for certain plants in the F_2 and F_3 generation of the cross *Franciscana* B × *biennis,* but I shall continue the lines further before reporting upon the results in full.

RECIPROCAL CROSSES BETWEEN *OENOTHERA BIENNIS* AND *OE. FRANCISCANA*

In a recent paper (DAVIS 1914, pp. 190-191) I have described reciprocal crosses between *biennis* and *Franciscana* and contrasted their characters in parallel columns. These details need not be presented again and I shall give here only a brief summary of the chief points of distinction together with a set of figures illustrating many of these features.

The mature rosettes of the reciprocal crosses are contrasted in figures 7 and 8. That of *Franciscana* × *biennis* (figure 7) has large leaves, broadly elliptical or spatulate, and strongly crinkled. Clearly a blend of parental characters (compare figure 7 with figures 1 and 2), there are, however, some patroclinous tendencies, although not conspicuous. The mature rosette of *biennis* × *Franciscana* (figure 8) presents leaves much

FIGURE 9.—Mature plant of F_1 hybrid, 13.35 *ac, Franciscana* B × *biennis.* Patroclinous in habit and foliage. Compare with figure 3, *biennis,* and contrast with figure 10, *biennis* × *Franciscana* B.

less crinkled and narrower than in the reciprocal (figure 7) to which it presents a sharp contrast; it is more strongly patroclinous (compare figure 8 with figure 2).

The mature plants are readily distinguished in habit and foliage. Those of *Franciscana* × *biennis* (figure 9) show the *biennis* habit of side branches from the rosette almost equal to the central shoot (compare with figure 3); the leaves (figure 11), although in form intermediate between the parents, are relatively broad. The plants of *biennis* × *Franciscana* (figure 10) resemble *Franciscana* in having side branches from the rosette much shorter than the central shoot (compare with figure 4); the leaves (figure 12) are narrower than in the reciprocal. Thus both crosses in habit and foliage are sharply distinguished and both, inclining towards the pollen parent, show patroclinous tendencies.

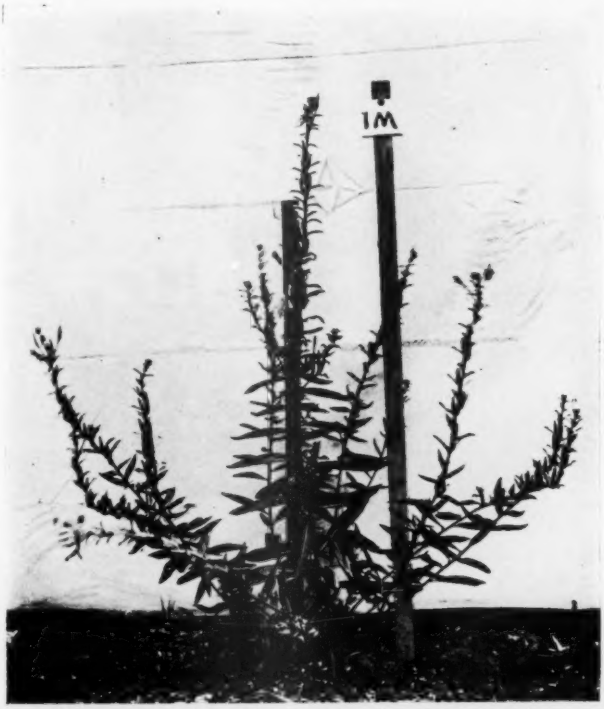

FIGURE 10.—Mature plant of F_1 hybrid, 13.36 *a*, *biennis* × *Franciscana* B. Patroclinous in habit and foliage. Compare with figure 4, *Franciscana* B, and contrast with figure 9, *Franciscana* B × *biennis*.

The pubescence of the stem (pilose and puberulent), although similar in kind in the two reciprocals, differs markedly in amount, the hybrids being in this respect also patroclinous. Only in the fact that the papillae over green portions of the stem are bright red in both hybrids do we find agreement and this is a very striking fact which would indicate dominance of this character of *Franciscana* over its absence in *biennis*.

FIGURE 11.—Inflorescence of F, hybrid, 14.37 *ac*, *Franciscana* E × *biennis*. Patroclinous in the broader bracts and slender sepal tips. At the right is a leaf from near the base of the main stem. Compare with figure 5, *biennis*, and contrast with figure 12, *biennis* × *Franciscana* E.

The inflorescence and buds of the reciprocal crosses likewise show distinctive features partially shown in figures 11 and 12. In their breadth the bracts incline towards the pollen parents. The buds of *Franciscana* × *biennis* (figure 11), 6.5—7 cm long, are intermediate in

size between the parents, but in the slender form of the sepal tips and in their less heavy pubescence they resemble more closely *biennis*. The buds of *biennis* × *Franciscana* (figure 12), also 6.5—7 cm long, resemble *Franciscana* in the strongly 4-angled cone, thick sepal tips and very heavy pubescence. The sepals in both crosses are streaked with red in varying depths of color and the ovaries bear red papillae so that

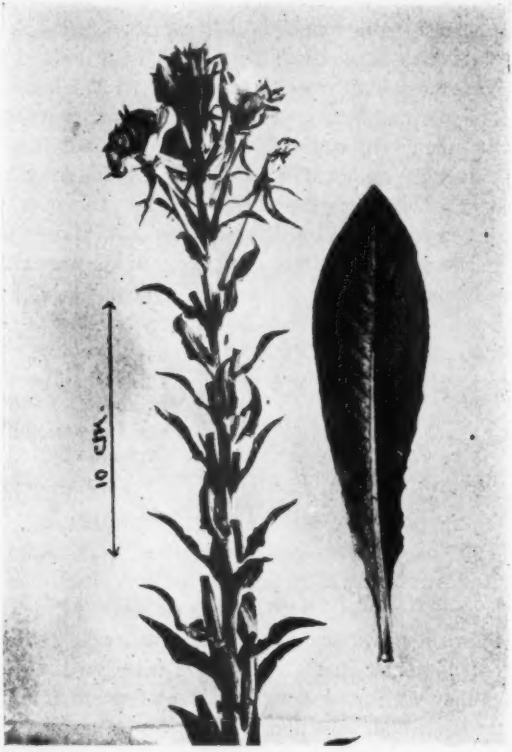

FIGURE 12.—Inflorescence of F_1 hybrid, 14.38 *a*, *biennis* × *Franciscana* E. Patroclinous in the narrower bracts and thick sepal tips. Compare with figure 6, *Franciscana*, and contrast with figure 11, *Franciscana* E × *biennis*.

again a color character present in *Franciscana* appears to dominate over its absence in *biennis*.

The petals offer points of difference in size and color too small for consideration in view of the variations present even upon the same plant, but the size, 2.1—2.5 cm long, is close to that of *biennis*.

The position of the stigma lobes, near the level of the anther tips, is intermediate between the conditions in close-pollinated *biennis* and open-pollinated *Franciscana;* the lobes, 6—7 mm long, are longer than in either parent.

The capsules of *Franciscana* × *biennis,* 2—2.8 cm long, are shorter and have a less heavy pubescence than in *biennis* × *Franciscana,* 2.2—3 cm long. In these respects the reciprocal crosses are also patroclinous, but these characters are intermediate between the parents.

We have noted that there are patroclinous tendencies expressed in a variety of characters of the reciprocal crosses, but I have emphasized the fact (Davis 1914) that a *morphological* character appears never to be passed on to the F_1 hybrids exactly as it is found in either parent. Even where the morphological resemblance to one of the parents is strongest I have always found evidence of the influence of the other species. With respect to the red pigmentation of the papillae on the stems and ovaries, and of the red coloration of the sepals the presence of this color character in both reciprocals indicates as noted above the dominance in this respect of *Franciscana* over *biennis.* The hybrids proved to be matroclinous as to the time of appearance of the side branches from the rosettes relative to the growth of the central shoot.

HYBRIDS OF THE CROSS *FRANCISCANA* E × *BIENNIS* IN THE SECOND GENERATION, AFTER COMPLETE GERMINATION OF THE SEEDS,—CULTURE 15.37

An F_1 plant, 14.37 *ac* (figures 7 and 11), representative of the cross *Franciscana* E × *biennis* furnished the seed for this culture. The contents of 6 capsules were sown on January 30 in a Petri dish. Germination ran for about 8 weeks (until March 29) and gave 513 seedlings. On April 8 the residue was examined and 450 sterile seeds were found to be empty of contents; in addition there were 70 small undeveloped structures together with ovule powder; 19 seeds were found containing small amounts of cellular contents. Thus from a sowing of about 982 (513+450+19) seed-like structures 513 seedlings appeared—a germination of 52 percent.

Observations on delayed germination of seeds sown in earth were made on another culture from the same parent plant 14.37 *ac.* The sowing of 396 seed-like structures from 4 capsules was made on January 8, 1915, in sterilized soil and the seedlings picked off and counted as they appeared. After 8 weeks (March 5) the culture had yielded 86 seedlings,—a germination of 21.7 percent. After 14 weeks (April 15) the

number of seedlings totaled 111,—a germination of 28 percent. With 18 weeks (May 15) the number had risen to 164 seedlings,—a germination of 41 percent. After 25 weeks (July 1) the culture had produced 192 seedlings,—a germination of 48.4 percent. During the summer 2 more seedlings appeared, and in February, 1916, still another. The seed-pan was emptied 16 months after the sowing of January 8, 1915. In all 195 seedlings appeared,—a germination of 49 percent. It required 25 weeks to give results accomplished in the Petri dish before 8 weeks. The worker content to accept the result of 8 weeks in the seed-pan would in this case obtain germinations from less than ½ of the viable seeds. The results point a sharp criticism of the past methods of genetical research among the oenotheras indicating how fragmentary may have been the cultures upon which ratios have been figured and conclusions have been based.

There was a high mortality among the 513 seedlings and young plants of this culture in comparison with other cultures from seeds germinated experimentally and I had only 466 rosettes at the time when the culture was set out in the garden. These ⅓ grown rosettes while in pots fell clearly into groups and were planted as follows:

20 rosettes with leaves narrower than in *Franciscana*,
204 rosettes *Franciscana*-like,
40 rosettes *biennis*-like,
54 rosettes intermediate between *biennis* and *Franciscana*,
24 rosettes with leaves strongly obtuse-pointed,
107 rosettes, dwarf and weak, with sickle-shaped leaves,
17 rosettes of weak dwarfs and backward plants which could not be placed in the groups above.

Some of these rosette peculiarities were later found to be correlated with characters of the mature plants and thus gave evidence of a prospective segregation of the culture. As the young rosettes grew to full size in the garden some of them changed their characteristics, thus rendering necessary a modification of the above count made at the time when the plants were set out in the open. These changes affected chiefly the 54 rosettes intermediate between *biennis* and *Franciscana* which generally came to resemble more closely one or the other of the parents.

The culture at maturity presented an assemblage which could be readily analyzed for a few characters but was extremely complex for others. My observations were centered on (1) the mature rosette, (2) the presence or absence of red coloration in the papillae of the stem and

ovaries, (3) the character of the foliage whether *biennis*-like or *Franciscana*-like in form, color and texture, (4) the form of the bud tips, (5) size of the petals, (6) the position of the stigma, whether above or below the tips of the anthers, (7) the type of capsule. The form of rosettes, coloration of papillae, character of foliage, form of bud tips, and type of capsule definitely followed certain principles of correlation. The size of petals and position of stigma followed no fixed law of association that I could discover. In general *biennis*-like plants had small flowers and *Franciscana*-like plants large flowers but there were numerous exceptions to this association and also many plants bore flowers in varying degrees intermediate between the parents.

In the tables that follow I shall designate certain characters as *biennis*-like or *Franciscana*-like, but this will not mean that the parental characters are always exactly duplicated. It will, however, signify resemblances so strong that there could be no hesitation in separating the plants under examination and furthermore the classes were without connecting intermediate forms. These characters generally appeared somewhat modified but there were cases where they were indistinguishable from those of the parent species. The study convinced me that a process of segregation determined the results exhibited by the culture and thereby illustrated the fundamental principles of Mendelian inheritance, i.e., segregation in the F_2 generation.

I shall lay no emphasis on the ratios presented by the results since the data is obviously imperfect for two reasons. First, there was a large mortality, especially among the dwarfs and groups of weaker plants which for the most part died before reaching maturity. Second, in a cross with so small a proportion of viable seeds (52 percent) and where the pollen of both parents is also partially sterile there is open the possibility of the elimination of large numbers and perhaps even classes of segregates the absence of which would very seriously complicate the situation.

The field notes which I took on the culture after the rosettes had reached maturity, at the time of flowering, and later, led to the arrangement presented in the following table. If this is compared with the list of groups as set out in the garden two important changes will be noted. First, the group of 54 rosettes previously listed as intermediate between *biennis* and *Franciscana* has disappeared because most of these plants later took on characters *biennis*-like although some became *Franciscana*-like. Second, a group of plants is noted in which the central shoot developed at once from an evanescent rosette. This group comprised plants

TABLE 1

The chief segregates in culture 15.37, the F_2 of Franciscana E × biennis.

Mature rosettes	Papillae on stems and ovaries	Foliage at maturity	Bud tips	Capsules
Leaves narrower than *Franciscana* 16	Red 9	*Franciscana*-like in color and texture, but narrower-leaved 9	*Franciscana*-like 5	*Franciscana*-like 2
Franciscana-like 182	Red 177	*Franciscana*-like 177	*Franciscana*-like 174	*Franciscana*-like 171
biennis-like 67	Red 63	*biennis*-like 63	*biennis*-like 62	*biennis*-like 62
Central shoot developing at once from an evanescent rosette 35	Red 33	*Franciscana*-like 29 *biennis*-like 4	*Franciscana*-like 25 *biennis*-like 4	*Franciscana*-like 19 *biennis*-like 4
Leaves strongly obtuse-pointed. Rosette evanescent 24	Green 23	*Franciscana*-like in color and texture, but broader-leaved 23	*Franciscana*-like 23	*Franciscana*-like 23
Dwarfs, leaves sickle-shaped 59	Red 39	*Franciscana*-like 36 *biennis*-like 3	*Franciscana*-like 36 *biennis*-like 3	*Franciscana*-like 30 *biennis*-like 3
Other dwarfs which died as rosettes 12				
Totals 395	344	344	332	314

some of which appeared among those previously listed as *Franciscana*-like and *biennis*-like, but also included some previously listed as dwarfs.

Before considering such data as is presented in the table above it will

be well to note how great was the mortality of the culture as a whole after the germination of the seeds, and also the much reduced number of plants upon which observations could be made with respect to the color of papillae, foliage, bud tips, and capsules. It will be remembered that the germination gave 513 seedlings of which only 466 produced rosettes in the hot-house. The number of rosettes that reached maturity in the garden amounted to only 395, the mortality naturally being greatest among the dwarfs although occurring also in other groups. The number of plants that sent up shoots upon which observations could be made respecting the color of papillae and character of foliage was 344, the number of plants that produced buds was 332 and capsules were developed upon 314 plants. Thus of 513 seedlings only 314 completed the life cycle, the remaining 199, either because of weak constitution or through accidental death, failed to reach full maturity. This mortality or failure to reach full maturity does not signify unfavorable garden conditions; it is the expression of phenomena that I have found characteristic of this cross and very general among species hybrids of Oenothera. It means that immense numbers of zygotic combinations are constitutionally defective or physiologically weak.

Taking the data as they stand there are, however, some very interesting correlations of characters, the most important of which will be noted in the following paragraphs.

The behavior in the F_2 of the color character of red in the papillae on stems and ovaries furnished an illustration in the culture, of the segregation of a character present in one parent, *Franciscana,* and absent in the other species, *biennis.* The character was dominant in the F_1 generation and consequently in the F_2 there might be expected a class of recessives with green stems and ovaries. Such green plants appeared and were wholly confined to the group with evanescent rosettes the leaves of which were strongly obtuse-pointed. Careful search on these plants failed to disclose any red papillae. All other plants of the culture presented red papillae, even those *biennis*-like in other respects, but the numbers of papillae distributed over the stems and ovaries varied greatly with different individuals. The ratio of green plants (23) to those with red papillae (321) is as 1:14, but in view of the pollen- and seed-sterility together with the high mortality in the culture, I lay no stress on this ratio which some geneticists might hail as significantly close to the 1:15 ratio. It is, however, proper to emphasize the fact that there is here presented a clean-cut segregation, Mendelian in its essential features.

A surprising feature of the culture at maturity was its sharp division

into two groups of plants which, with respect to foliage, bud tips, and capsules, resembled either the *Franciscana* or the *biennis* parent. The distinctions between the groups was so clear that no difficulty was experienced in separating the plants. Those *Franciscana*-like had a foliage of narrower, thicker leaves of a darker shade of green, thicker sepal tips, and longer capsules. Those *biennis*-like presented a foliage of broader leaves more thin in texture and lighter in color, sepal tips slender, and shorter capsules. There was generally a heavier pubescence on plants *Franciscana*-like and they were usually taller than those which resembled *biennis*. A record of the foliage characters at maturity showed 274 plants resembling *Franciscana* and 70 *biennis*-like.

Within the *Franciscana*-like group there was a remarkable range of flower-size and a varied position of the stigma whether high or low, i.e., whether above or below the tips of the anthers. There is presented in table 2 the data for 219 of the 274 plants *Franciscana*-like in foliage, a record imperfect because of the slowness or failure of many dwarfs to flower. Table 2 shows clearly a tendency towards the association within the same flower of large petals and a high position of the stigma, both characters of *Franciscana*. It likewise shows a less definite association of small petals with the low position of the stigma, both characters of *biennis*. Petals intermediate in size between the parents are more likely to have a stigma *Franciscana*-like in position. It is important to note that 52 plants with large flowers and high stigmas also resembled *Franciscana* so strongly in their rosettes, red papillae, foliage, bud tips, and capsules as virtually to constitute a class of *Franciscana*-like segregates. The differences between them and the parent species were small plus and minus expressions of the characters of *Franciscana*. In the

TABLE 2

Position of stigma relative to the size of petals on plants Franciscana-like in foliage; culture 15.37, the F_2 of Franciscana E × biennis.

Petals	Stigma high (*Franciscana*-like)	Stigma low (*biennis*-like)	Stigma intermediate	Totals
Large (*Franciscana*-like)....	52		2	54
Small (*biennis*-like).....	11	49	55	115
Intermediate	29	1	8	38
Smaller than *biennis*.....		7	5	12
Totals.....	92	57	70	219

group of 12 plants with flowers smaller than *biennis* were 3 individuals with petals 9 mm long and crumpled.

Within the second group, *biennis*-like as to foliage, there was likewise a wide range of flower-size and a varied position of the stigma whether high or low. The data for 64 of the 70 plants of this group is presented in table 3. It will be seen that of the 7 large-flowered plants only one had stigmas in the low position. There was a clear tendency for plants with small flowers to be also *biennis*-like in the position of the stigma. Flowers intermediate in size between the parents showed no marked tendency as to the position of the stigma. In the group of 64 plants there were 33 plants essentially indistinguishable from the *biennis* parent in their rosettes, bud tips, flowers, and capsules as well as in foliage. They would have formed a class of *biennis*-like segregates except for the fact that all had red papillae on their stems and ovaries, although on some plants the number of red papillae was small. The culture thus furnished no illustration of a complete segregation of *biennis* characters since the 23 plants with green papillae were all in a group *Franciscana*-like in foliage, bud tips, and capsules (see table 1).

Finally, the large group of dwarfs should be briefly considered. Almost all of these were recognized when young rosettes by their slower growth and by the fact that their leaves were generally asymmetrical or sickle-shaped, as illustrated in figure 18. Although fully green in color they were vegetatively weak from the beginning. Some died in the hot-house and many others failed to survive the conditions of the garden where a slight degree of drought proved to be fatal. Of the 107 dwarf rosettes with sickle-shaped leaves which were set in the garden only 39 produced shoots. Some of these became fair sized but most of them reached a stature of not more than 2—4 dm, having much the appearance shown in figure 19. Of the 39 plants which reached maturity 36 were *Franciscana*-like in foliage and bud tips, while only 3 presented the aspects of *biennis*. From the fact that some dwarf rosettes produced fair-sized plants and thus largely outgrew their prospective condition, and also because a few dwarfs appeared in groups originally composed of strong rosettes it is possible that their weakness may be the effects of a diseased condition rather than due to a specific genetical constitution. This point must be tested by further experimentation to establish conclusions.

TABLE 3

Position of stigma relative to the size of petals on plants biennis-like in foliage; culture 15.37, the F_2 of Franciscana E × biennis.

Petals	Stigma high (*Franciscana*-like)	Stigma low (*biennis*-like)	Stigma intermediate	Totals
Large (*Franciscana*-like)....	5	1	1	7
Small (*biennis*-like).....	1	34	4	39
Intermediate	5	5	5	15
Smaller than *biennis*.....		3		3
Totals.....	11	43	10	64

HYBRIDS OF THE CROSS *BIENNIS* × *FRANCISCANA* E IN THE SECOND GENERATION, AFTER COMPLETE GERMINATION OF THE SEEDS,—CULTURE 15.38

This culture came from an F_1 plant, 14.38 *a*, (figures 8 and 12) of the cross *biennis* × *Franciscana* E, the exact reciprocal of the plant, 14.37 *ac*, which gave the F_2 generation described in the previous section. Sowings on January 30 of the contents of 2 capsules in a Petri dish gave after 3½ weeks (February 24) 322 seedlings. No other germinations appearing, the residue (figure 13) was examined on March 10 and found to contain about 240 sterile seeds empty of contents and in addition about 80 small undeveloped structures together with ovule powder (figure 13). Thus from a sowing of about 562 (322 + 240) seed-like structures 322 seedlings developed,—a germination of 57 percent.

Another comparative test of delayed germination of seeds sown in earth was made in a culture from the same parent plant, 14.38 *a*. A sowing of 367 seed-like structures from 2 capsules was made on January 8, 1915, in sterilized soil. After 8 weeks (March 5) 104 seedlings had appeared,—a germination of 28 percent. After 14 weeks (April 15) the culture had yielded 187 seedlings,—a germination of 50.9 percent. With 18 weeks the number of seedlings had risen to 200,—a germination of 54 percent. After 25 weeks (July 1) the culture produced in all 243 seedlings,—a germination of 66 percent. During the summer 3 seedlings appeared and in the autumn 4 more. The pan was emptied 16 months after the sowing of January 8, 1915. In all 250 seedlings were obtained,—a germination of 68 percent, which is somewhat higher than that obtained in the Petri dish (57 percent) and it is of interest to note

that both cultures were from sowings of 2 capsules each, but that in the Petri dish consisted of about 562 while that in the earth was of 367 seed-like structures. The smaller number of seed-like structures in a capsule in this case appeared to result in a higher percentage of viable seeds. As illustrated in the case of the culture previously described a worker content to accept the germination from 8 weeks in the seed-pan would have obtained a germination from less than ½ of the viable seeds. So again, the method of experimental and complete germination of seeds justifies the criticism which I offer of past methods of genetical work among the oenotheras.

The mortality among the seedlings of this culture was very much less than in the culture previously described since from 322 seedlings 302

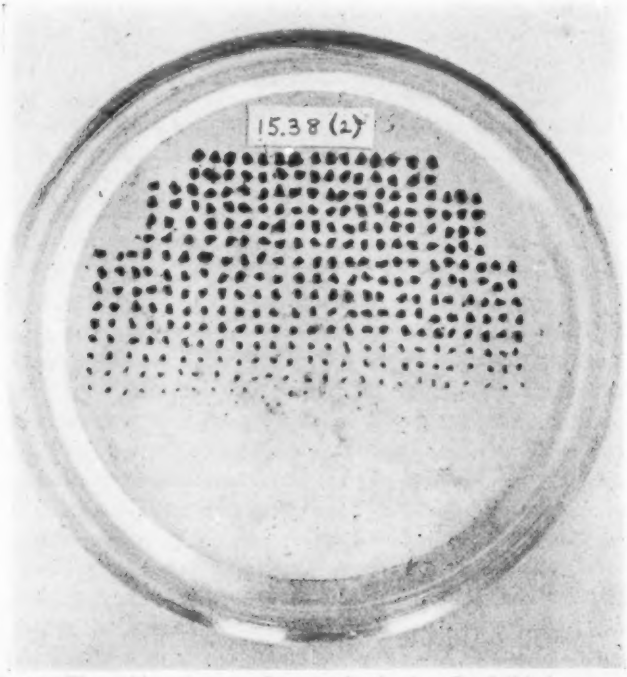

FIGURE 13.—The residue after complete germination in a Petri dish from a sowing of about 562 seed-like structures, culture 15.38, F_2 of *biennis* × *Franciscana* E. The germination produced 322 seedlings and there was left, as the residue, about 240 empty structures the size of seeds and about 80 structures of smaller dimensions which graded into ovule powder.

rosettes developed in the hot-house. The ⅓-grown rosettes were planted in the following groups:

16 rosettes with leaves narrower than in *Franciscana,*
236 rosettes *Franciscana*-like in form but frequently broader-leaved,
44 rosettes dwarf and weak, with sickle-shaped leaves,
6 rosettes of weak dwarfs which could not be placed in the groups above.

There was the usual modification of characters in some of the rosettes as they came to maturity, rendering necessary a reclassification and modification of the above counts made at the time when the plants were set in the ground. A very large mortality among the rosettes lowered the number which reached maturity from 302 to 247, and of the latter only 208 survived to send up shoots. The mortality was greatest among the dwarfs but also affected rosettes that were *Franciscana*-like.

With the appearance of the shoots the resemblance of the culture as a whole to *Franciscana* became more marked. The foliage at maturity was wholly of the *Franciscana* type in its darker green color, thicker texture and in leaf form although the leaves were generally somewhat larger. The papillae on stems and ovaries were red in all plants as in *Franciscana.* The bud tips were all *Franciscana*-like and to a lesser degree the capsules. There was, therefore, in the culture no group of plants with wholly green stems and ovaries and no plants with foliage, bud tips and capsules *biennis*-like. There was, however, a wide range of variation in the flower size and in the position of the stigma whether high or low. My notes on the culture after the rosettes had become full sized and later when the plants reached maturity are presented in table 4 which may readily be compared with table 1.

Although I have grouped the plants in table 4 as *Franciscana*-like in most respects, this must not be understood to mean a perfect duplication of the parent in these characters. Leaves were generally larger than in *Franciscana* and capsules were frequently somewhat smaller, while bud tips varied in their length. Moreover, in flower size and in the position of the stigma the culture, *Franciscana*-like in other respects, presented many plants with the characters of *biennis.* There were, nevertheless, 6 plants in the culture essentially indistinguishable from *Franciscana* with respect to all of the characters discussed in this paper, the differences being small plus or minus expressions of the characters concerned.

The absence in this culture of a group of plants *biennis*-like in foliage, bud tips, and capsules was a surprise as was also the absence of green-stemmed plants representing a group of recessives from the dominant

TABLE 4

The chief segregates in culture 15.38, the F_2 of biennis × Franciscana E.

Mature rosettes	Papillae on stems and ovaries	Foliage at maturity	Bud tips	Capsules
Leaves narrower than *Franciscana* 17	Red 6	*Franciscana*-like in color and texture but narrower-leaved 6	*Franciscana*-like 4	*Franciscana*-like 4
Franciscana-like 186	Red 172	*Franciscana*-like 172	*Franciscana*-like 170	*Franciscana*-like 160
Intermediate between *biennis* and *Franciscana* 26	Red 24	*Franciscana*-like 24	*Franciscana*-like 20	*Franciscana*-like 17
Dwarfs, leaves sickle-shaped 15	Red 6	*Franciscana*-like 6	*Franciscana*-like 6	*Franciscana*-like 4
Other dwarfs which died as rosettes 3				
Totals 247	208	208	200	185

character of red papillae present in the F_1 generation. The only conspicuous suggestions in the culture of *biennis* characters were found in those rosettes intermediate in character between the parent types and in the flowers *biennis*-like as to the size of their petals and the position of their stigmas. I have no hypothesis to account for this one-sided expression of the culture so suggestive of *Franciscana,* but it should be emphasized that the high mortality in the culture, the large proportion of sterile seeds, and the partial sterility of the pollen indicate the probability of a very imperfect expression of the segregation which theoretically might be expected from the zygote of the F_1 parent plant. Until the significance of the gametic and zygotic sterility as well as the high mortality among the plants is understood it would be rash indeed to assume that segregation had not taken place during the development of the gametes at the end of the first hybrid generation. Therefore, that

which came to maturity in my cultures may have been from but a part of the range of zygotes formed and only a partial representation of the types theoretically possible if all of the gametes had been fertile among themselves.

TABLE 5

Position of stigma relative to the size of petals on plants which as rosettes were Franciscana-like; culture 15.38, the F_2 of biennis × Franciscana E.

Petals	Stigma high (*Franciscana*-like)	Stigma low (*biennis*-like)	Stigma intermediate	Totals
Large (*Franciscana*-like)	6			6
Small (*biennis*-like)	6	33	17	56
Intermediate	14	3	11	28
Smaller than *biennis*	3	1	2	6
Totals	29	37	30	96

The distribution of flower types on plants from rosettes which were *Franciscana*-like is shown in table 5. It will be seen that only 6 plants had large flowers and these also presented a high position of the stigma. Of 28 plants with petals intermediate in size between the parents only 3 had the stigma in a low position, and of the 56 plants with small flowers only 6 presented the stigma in the high position. The tendency, therefore, was for an association in the same flower of large petals with high stigmas and of small petals with low stigmas, but there were exceptions to this correlation.

TABLE 6

Position of stigma relative to the size of petals on plants which as rosettes were intermediate between biennis and Franciscana; culture 15.38, the F_2 of biennis × Franciscana E.

Petals	Stigma high (*Franciscana*-like)	Stigma low (*biennis*-like)	Stigma intermediate	Totals
Large (*Franciscana*-like	1		2	3
Small (*biennis*-like)		10	1	11
Intermediate		4	1	5
Smaller than *biennis*		1		1
Totals	1	15	4	20

On 20 plants from rosettes intermediate between *biennis* and *Franciscana* (table 6), the flowers were mostly small or intermediate in size and the position of the stigma was generally low. Only 1 plant had large petals together with a high position of the stigma.

The group of dwarfs with sickle-shaped leaves was large and early recognized. They were similar to the dwarfs described in the previous culture and like them were vegetatively very weak. Only 6 out of the 44 produced shoots. I noticed in the development of the culture that many plants showed tendencies towards the production of sickle-shaped leaves, tendencies which were later outgrown, and this leads me to suggest that this dwarf form, as in the previous culture, may be the result of a diseased condition. It is a matter for further study.

HYBRIDS OF THE CROSS *FRANCISCANA* B × *BIENNIS* IN THE SECOND GENERATION, FROM SEEDS GERMINATED IN THE EARTH

My observations on the F_2 hybrids of this cross, *Franciscana* B × *biennis*, were made in the summer of 1914, a year before the method of experimental seed-germination was developed. The cultures were from sowings in the earth, the seed-pans being kept for 8—11 weeks. There is, therefore, no assurance that all of the possibilities of the cultures were fully represented, since delayed germination undoubtedly held back a large part of the progeny. Consequently, the observations have little value as a genetical study of segregation and are only described because there were found in these cultures some interesting variants suggestive in certain respects of the so-called "mutants" from *Lamarckiana*. In this behavior of throwing occasionally exceptional forms these cultures presented a marked contrast to the F_2 from the cross *Franciscana* E × *biennis*, where, as we have seen, there was a clearly defined grouping of the types into a few large classes. From this difference in behavior when mated to the same line of *biennis* I am inclined to believe that the race *Franciscana* B is not so pure as the race *Franciscana* E. The cultures had their chief value as marking an important advance in the problem of synthesizing a *Lamarckiana*-like hybrid from a race of *Franciscana* pollinated by *biennis*, an account of which will be published at a later time.

The F_1 generation, 13.35, from which the cultures came, consisted of 165 plants and has already been described in detail (DAVIS 1914, pp. 188-192). It was more variable than that from the cross *Franciscana* E × *biennis* and five sister plants were selected for the parents of the five

selfed lines constituting the F_2 cultures, because of certain individual peculiarities as follows:

(1) Plant 13.35 *ac*, a type with smaller leaves, smaller flowers (petals 2 cm), and shorter stature than some plants of the culture. From this parent the F_2 culture 14.51 was grown, consisting of 386 plants.

(2) Plant 13.35 *b*, similar to plant 13.35 *ac* except for somewhat broader leaves and slightly larger flowers (petals 2.1 cm), the parent of the F_2 family 14.52 consisting of 255 plants.

(3) Plant 13.35 *c*, one of the largest-flowered types in the culture (petals 2.4 cm) and also large-leaved, the parent of an important F_2 culture 14.53 of 363 plants.

(4) Plant 13.35 *g*, similar to 13.35 *c* but with sepals at mid-summer entirely green, later taking on reddish streaks. From this parent came the F_2 culture 14.54 numbering 211 plants.

(5) Plant 13.35 *r* with sepals very red as in *rubrinervis*, and the mid-veins of the rosette leaves also a deeper red than in the mass of the culture, also with larger flowers (petals 2.4 cm) and larger leaves. This strongly colored type graded through intermediates to plants with the minimum of red as in 13.35 *g*. From it was grown the F_2 family 14.55 with 421 plants.

As was to be expected the families in the F_2 from the five sister plants listed above were distinguished in some important respects although having certain features in common. The points of agreement will be considered first.

In the five families constituting the total F_2 progeny 1636 plants sent up shoots upon which observations could be made respecting the color of the papillae over green portions of the stems. On all of these plants red papillae were found; none of them had the clear green stems of the *biennis* parent. Such results might lead one to suppose that there had been no segregation of this character (red of papillae) present in *Franciscana* and absent in *biennis*. Luckily there was left after the sowing of 1914 the contents of 8 capsules from the plant 13.35 *ac*. I was thus able in 1915 to obtain complete germinations in Petri dishes and thus to test the possibility that a class of recessives might be represented in seeds that required a longer time in the earth for their germination than the 8 to 11 weeks allowed in 1914.

The sowings of 1914 from plant 13.35 *ac* (culture 14.51) in earth produced 402 seedlings from 819 seed-like structures,—a germination of 49 percent. The sowings of 1915 of seeds from the same plant in a Petri dish (culture 15.51) gave 761 seedlings from 921 seed-like struc-

tures,—a germination of 82.6 percent. Thus the experimental method of seed-germination increased the product from the sowing 33.6 percent. The culture of 1914 from seeds sown in the earth produced from 402 seedlings 386 plants with shoots. Unfortunately the culture of 1915 from seeds germinated in Petri dishes was started so late in the hot-house that very many of the plants refused during the summer to pass from the rosette stage. Only 497 of the 762 seedlings produced plants

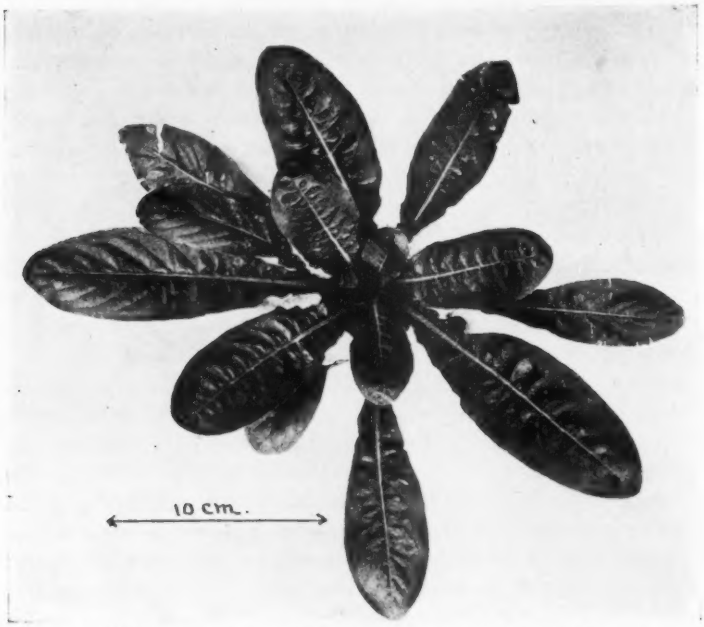

FIGURE 14.—Mature rosette of a plant, 14.51 *o*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B × *biennis*. Representative of a class distinguished by their rosettes of broad, obtuse-pointed leaves. Contrast with figure 16.

with shoots, but on 14 of these, a group of small-flowered types, I could find no red papillae. A group of green-stemmed plants thus appeared as in the case of the F_2 from the cross *Franciscana* E × *biennis* and again the experimental method of seed-germination justified itself as necessary for genetical research in the oenotheras.

The five families agreed closely as to the constitution of the mass of the cultures. There was present in each a well defined class distinguished as rosettes by their broad obtuse-pointed leaves (figure 14). Flowering

shoots from such rosettes presented a foliage of leaves and bracts also broad (figure 15) and small flowers of the *biennis* type with respect to the position of the stigma lobes below the level of the anther tips. The sepal tips were, however, thick and with a heavy pubescence like that of *Franciscana*. This class appeared in the five families in proportions of

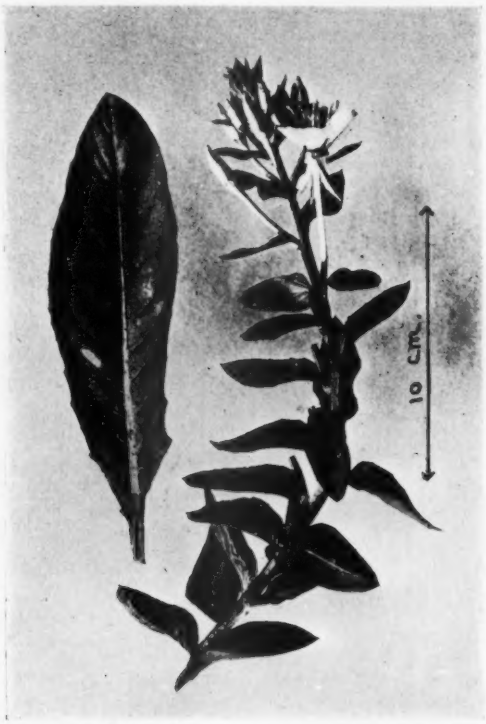

FIGURE 15.—Inflorescence of a plant, 14.51 *o*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B × *biennis*. Representative of a class with a foliage of obtuse-pointed leaves, broad bracts, and small flowers of the *biennis* type but with thick sepal tips as in *Franciscana*. At the left is a leaf from near the base of the main stem. Contrast with figure 17.

41 percent (culture 14.51), 32 percent (14.52), 34 percent (14.53), 32 percent (14.54), and 39 percent (14.55), and these figures at least show that the class was numerically large. Curiously this class was not represented in the F_2 from *Franciscana* E × *biennis* (see table 1) which

shows that *Franciscana* B has a different genetical constitution from *Franciscana* E in spite of its taxonomic similarity.

The remainder of the plants in the five families had rosette leaves acute-pointed (figure 16) although of varying width and the leaves of the flowering shoots were narrow (figure 17) and likewise acute-pointed. This was a complex assemblage exhibiting a wide range in the form and size of the leaves. The flowers also ranged from those similar to *biennis*

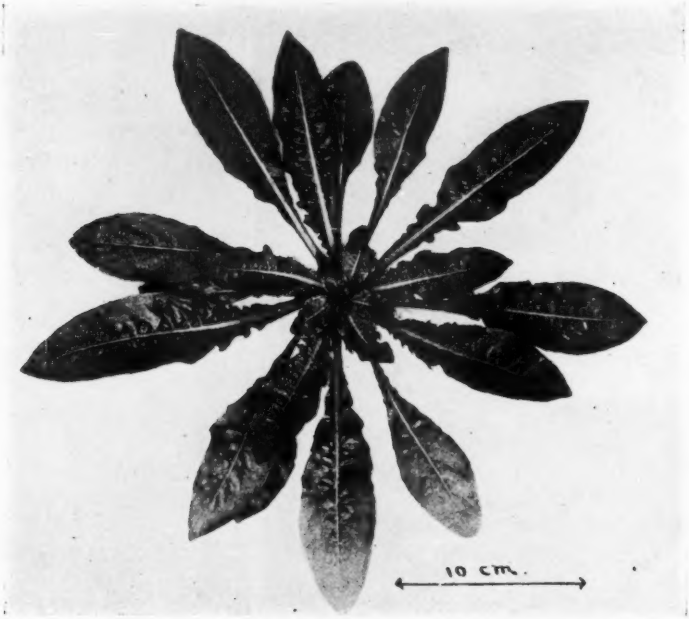

FIGURE 16.—Mature rosette of a plant, 14.51 *p*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B × *biennis*. Representative of a group with rosette leaves acute-pointed. Contrast with figure 14.

to those of the *Franciscana* type and the sepal tips on some plants were thick like those of *Franciscana* and in others long and slender as in *biennis* and *Lamarckiana*. The assemblage undoubtedly contained several distinct classes distinguished by the characters of the bud tips and flowers, but data were not taken properly to differentiate them. I have records of 7 plants essentially similar to the *Franciscana* parent and there were undoubtedly more, but none of the *biennis* type were found since clear green stems together with typical *biennis* foliage were not repre-

sented in the cultures. It was in this assemblage that plants resembling *Lamarckiana* appeared.

Among the plants with acute-pointed leaves there appeared in all five families the same type of dwarf which has been described for the F_2 generations from the reciprocal crosses between *biennis* and *Franciscana* E. These dwarfs could be recognized as half-grown rosettes by their asymmetrical, sickle-shaped leaves (figure 18). Such rosettes always developed small plants which sent up irregularly disposed side branches generally not more than 2—4 dm long (figure 19). The leaves were broadly elliptical, the sepal tips *Franciscana*-like, and the flowers small (petals 1—1.7 cm). These dwarfs were not numerous in the cultures,

FIGURE 17.—Inflorescence of a plant, 14.51 ♀, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B × *biennis*. Representative of a group with foliage of narrow, acute-pointed leaves and narrow bracts. At the right is a leaf from near the base of the main stem. Contrast with figure 15.

7 (14.51), 6 (14.52), 10 (14.53), 2 (14.54), and 3 (14.55), but it seems probable that a complete germination of the seeds would have given much larger proportions.

Some other types of dwarfs appeared but not uniformly in all of the five families. Culture 14.52 produced an *albida*-like plant and a very narrow-leaved dwarf both of which died early. In culture 14.53 were 3 small rosettes with leaves etiolated as in *albida* and a dwarf rosette with broader, obtuse-pointed leaves also etiolated; none of these lived

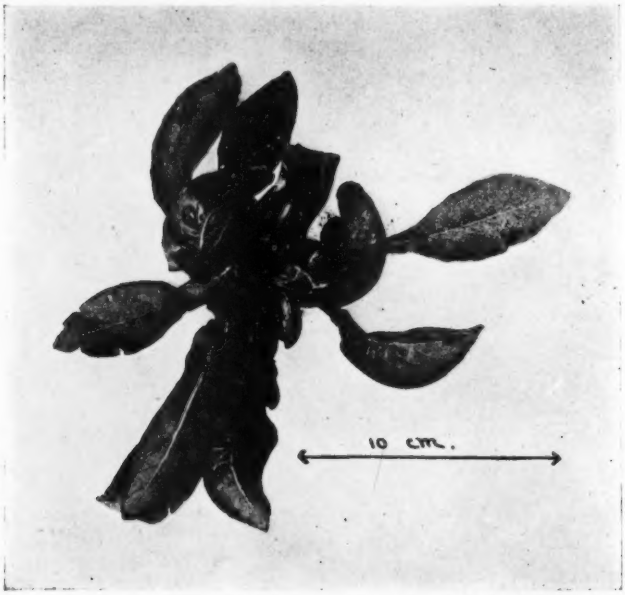

FIGURE 18.—Mature rosette of a dwarf with asymmetrical sickle-shaped leaves (culture 14.51). Representative of a group common in the F_2 generations from *Franciscana* × *biennis* and the reciprocal cross.

long. In culture 14.54 were 3 rosettes with leaves almost linear; they were fully green and lived through the summer but failed to produce shoots. In morphology these rosettes were very close to the figures and description of *Oenothera sublinearis* of DE VRIES, a "mutant" from *Lamarckiana* (Die Mutationstheorie, Vol. I, p. 285, 1901). A broad-leaved etiolated dwarf failed to develop beyond the rosette stage. Culture 14.55 had 9 etiolated dwarfs and a narrow-leaved plant all of which failed to survive the summer.

In the cultures was one plant, 14.51 *r*, worthy of special mention. Starting as a rósette with *Lamarckiana* characters it developed later a foliage of broad leaves (figure 20) but much shorter than in other broad-leaved forms in the cultures. The flowers were small (petals 1.1 cm) and in most cases the style and stigma withered to a brown filament rendering them infertile. This tendency towards the abortion of the style suggested the peculiarity of *brevistylis* although in other features

FIGURE 19.—Mature plant of a dwarf (culture 14.53) from a rosette with sickle-shaped leaves (figure 18). Representative of a group common in the F_2 generation from *Franciscana* × *biennis* and the reciprocal cross.

of its morphology there were no points of close resemblance to this plant. I was able to self a few flowers in which the style was not abortive and the progeny of this plant will be followed with interest.

One of the most interesting of the classes consisted of three plants

very conspicuous for their size and luxuriance. They are probably comparable to the triploid or *semi-gigas* forms from *Lamarckiana* (STOMPS 1912, LUTZ 1912), and *biennis* (STOMPS 1914, DE VRIES 1915 a), and also to the "hero" types from *Lamarckiana* or certain derivatives when pollinated by *leptomeres* (*biennis cruciata*), *muricata* or *Millersi* (DE VRIES 1913, p. 327). The plants of this giant or "hero" type appeared one in each of the cultures 14.52, 14.53, and 14.54. A description of the plant 14.52 *g* will serve for all. The extraordinarily large rosettes at maturity were 5.5 dm in diameter with leaves very broad and thick (figure 21). Side shoots from the rosettes (figure 22)

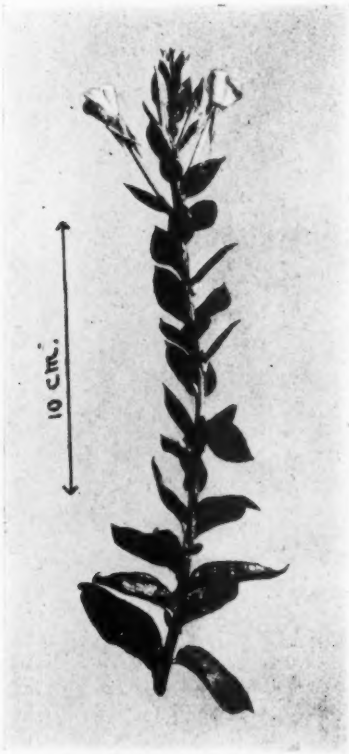

FIGURE 20.—Inflorescence of a plant, 14.51 *r*, in the F_2 from 13.35 *ac* (figure 9), *Franciscana* B × *biennis*. Peculiar for its foliage of short, broad leaves and for its small flowers in most cases with style and stigma withered to a brown filament rendering them infertile.

were unusually numerous (12 in 14.52 *g*, 11 in 14.54 *g*), widely spreading and very long (some 1—1.3 m). The stems were exceptionally strong, the leaves and bracts thick in texture. The buds (figure 23) were long (about 7.5 cm) and the cone stout although the flowers were not large (petals about 2.5 cm). The large stigma lobes were placed slightly below the level of the anther tips.

The evidence of gigantism exhibited by these plants suggested an increased number of chromosomes and a preliminary examination of mitotic figures in the developing ovules of plants 14.52 *g* and 14.54 *g* gave several counts certainly as high as 19 and 20 chromosomes so it seems probable that we have in these plants examples of triploidy (21 chromosomes). This view is further supported by the complete or al-

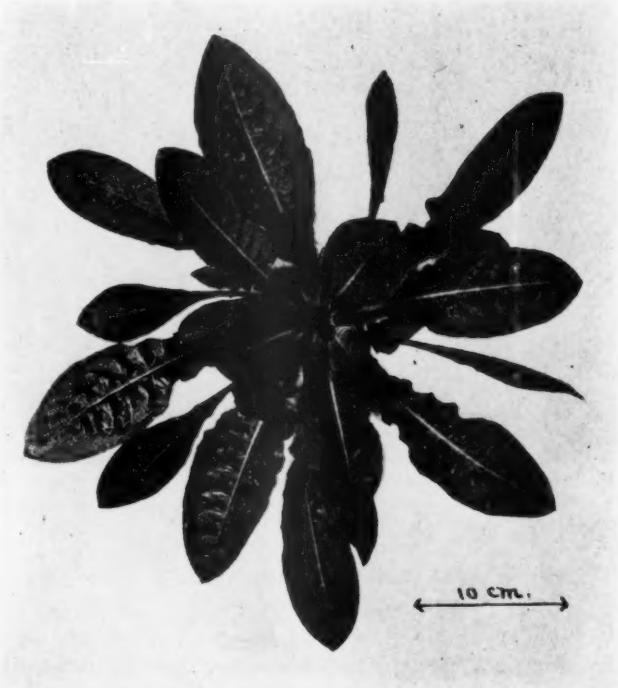

FIGURE 21.—Mature rosette of a plant, 14.52 *g*, in the F_2 from 13.35 *b*, *Franciscana* B × *biennis*. Remarkable for its very large, broad and thick leaves. Representative of three plants of a "hero" or *semi-gigas* type probably with the triploid number of chromosomes (21).

most complete self-sterility of the three plants, a condition characteristic of the triploid *semi-gigas* types that have been studied by other investigators. Attempts to self-fertilize numbered 21 for 14.52 *g*, 31 for 14.53 *g*, and 31 for 14.54 *g*. The yield was a few shriveled capsules containing some small structures of very doubtful viability which have not yet been sown. Attempts to pollinate the parent *biennis*, 28 from 14.52 *g* and 13 from 14.54 *g*, were all failures, and similar attempts to pollinate *Franciscana*, 11 from 14.52 *g* and 11 from 14.54 *g*, gave only a few

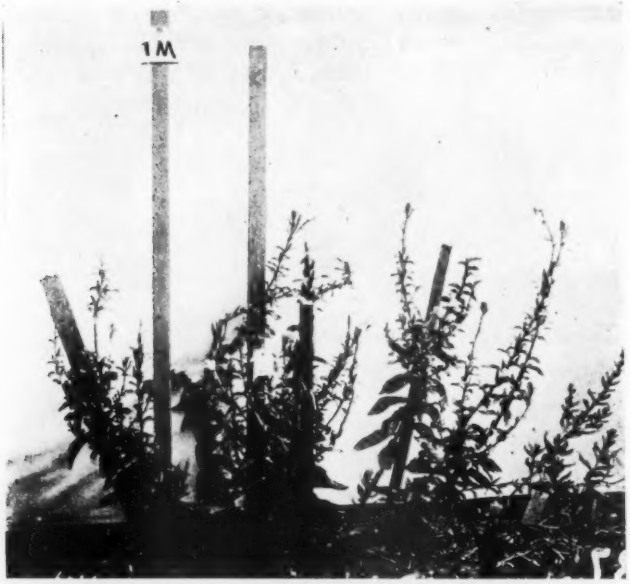

FIGURE 22.—Mature plant, 14.52 *g*, the *semi-gigas* type from the rosette shown in figure 21. Remarkable for its luxuriant growth as illustrated by the very long side shoots (some 1-1.3 m) from the rosette.

seeds(?) likely to prove sterile. In sharp contrast to the above experiments, flowers pollinated from the parents, *biennis* and *Franciscana*, developed capsules with a fair yield of apparently good seed, and open-pollinated flowers produced well filled capsules. Thus it appears clear that the self-sterility is not due to conditions within the ovules but must be concerned either with the development of pollen-grains, with a failure of pollen-tubes to reach the ovules, or to an inability of the gamete nuclei to fuse or to produce viable zygotes. A similar situation is pre-

sented in certain triploid hybrids of *gigas* with other oenotheras and offers an interesting cytological problem upon which I hope later to report.

There remain for consideration the plants which in certain respects resembled *Oenothera Lamarckiana.* They constituted a group selected at first because the mature rosettes (figures 24 and 25) closely matched those of *Lamarckiana,* and then observed throughout their development

FIGURE 23.—Inflorescence from the *semi-gigas* type, 14.52 *g*, (figures 21, 22), showing long buds with stout cones, but relatively small flowers (petals about 2.5 cm) with stigma lobes slightly below the level of the anther tips.

to establish whether or not as mature plants they continued to exhibit the peculiarities of this plant. Therefore with respect to the characters of the mature rosettes these plants constituted a group distinct from the obtuse-pointed, broad-leaved class (figure 14) and differing from other rosettes with acute-pointed leaves in having a leaf form and degree of

crinkling more close to that of *Lamarckiana*. The *Lamarckiana*-like rosettes were distributed among the five families as follows: 5 in 14.51, 1 in 14.52, 3 in 14.53, 2 in 14.54, and 13 in 14.55. These rosettes graded through intermediates into the large class with narrower-pointed leaves, illustrated by figure 16, which constituted more than one-half of the total number of plants in the cultures.

In culture 14.51, as stated above, there were 5 rosettes *Lamarckiana*-like in the form, size, and crinkled surface of their leaves. Of these plants 4 sent up shoots with a foliage also of the *Lamarckiana* type, but the flowers were all small (petals about 2 cm long) and the stigma lobes were close to the level of the anther tips, in some plants slightly above and in some slightly below. The 4 plants were of a type very similar to some of the small-flowered forms of *Lamarckiana* which I have obtained from seed of DE VRIES's (DAVIS 1912, p. 384) and from seed of English origin (DAVIS 1913 b, p. 237).

The single plant of culture 14.52, selected as being conspicuously *Lamarckiana*-like as a rosette, also proved at maturity to be a small-flowered type.

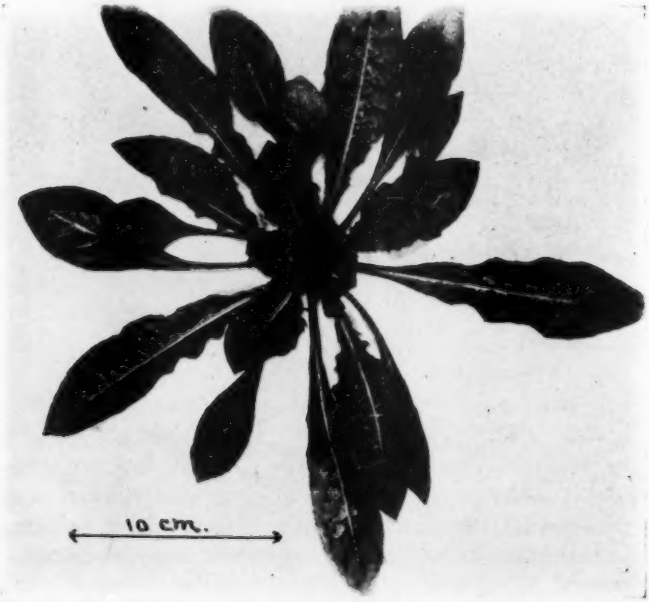

FIGURE 24.—A *Lamarckiana*-like rosette, plant 14.55 *ac*, in the F_2 from 13.35 *r*, *Franciscana* B × *biennis*.

Culture 14.53 gave what is likely to prove the most valuable of the *Lamarckiana*-like forms of the cross. A plant, 14.53 *c*, presented fairly large flowers (petals 3.3 cm) with stigma lobes 2—3 mm above the tips of the anthers and, what is very important for the synthesis of a *Lamarckiana*-like hybrid, short capsules (1.8—2 cm long), in form, size, and pubescence quite indistinguishable from those of *Lamarckiana*. As the plant stood in this F_2 generation it seemed to me to fall within the limits of DE VRIES's account of the variation present in *Lamarckiana*,

FIGURE 25.—A *Lamarckiana*-like rosette, plant 14.55 *c*, in the F_2 from 13.35 *r*, *Franciscana* B × *biennis*. Failing to send up the central shoot this rosette became unusually large.

although the plant did not quite match the largest-flowered races that he has distributed (petals 4—4.5 cm), races which appear to be the élite among the *Lamarckianas*. Among the progeny of this selfed plant grown last summer (1915) in the F_3 I have had types with larger flowers (petals 3.7 cm) and in all other important respects essentially similar to *Lamarckiana*. These will be described in detail probably after

the F_4 has been grown. The two other plants of culture 14.53, *Lamarckiana*-like in foliage, proved to be small-flowered.

The two *Lamarckiana*-like rosettes in culture 14.54 failed to send up shoots and did not survive the winter.

The 13 *Lamarckiana*-like rosettes of culture 14.55 fell into two groups. In 6 of these rosettes the mid-veins were white as is true of the races of *Lamarckiana* familiar to me; in 7 of the rosettes the mid-veins were reddish as described by HERIBERT-NILSSON (1912, 1915) for certain material that he has handled. The presence in the culture of white-veined rosettes is of interest for the reason that the rosette leaves of both *biennis* and *Franciscana*, as far as I have observed, always show more or less red pigmentation in their mid-veins, as was also true of their F_1 hybrids. The amount of red pigmentation varied greatly among the plants of the F_2 generations and I very much doubt whether the character could be followed with precision in the hybrids of my cultures.

Of the 6 *Lamarckiana*-like rosettes with white mid-veins, 4 sent up side branches that flowered and the foliage and bracts of these shoots (figure 26) presented the characters of *Lamarckiana*. Since no central shoots developed, the rosettes grew to be very large (figure 25) evidently adopting a biennial habit, as *Lamarckiana* frequently does under certain conditions. When the plants came to bloom, 3 bore large flowers (petals 3.5 cm) with the stigma lobes 3 mm or more above the tips of the anthers; the other plant was small-flowered (petals 1.5 cm) and the stigma lobes were 1—2 mm below the anther tips. The larger-flowered plants (figure 26) were among the most beautiful in the cultures. Their foliage was very close to that of *Lamarckiana* and the pubescence was similar, but the buds proved to be more slender, the inflorescence less compact, and the capsules (2.5 cm) too long and attenuate to match the type of *Lamarckiana* known to us from seeds of DE VRIES, although the capsules fell both as to form and size within the range of variation given by him ("Die Mutationstheorie", Vol. I, pp. 337—339, figure 114, 1901). I have grown an F_3 generation from one of these plants and while I obtained larger flowers (petals 4 cm) and a type of inflorescence more like that of *Lamarckiana* there were no plants with short capsules.

Of the 7 *Lamarckiana*-like rosettes with red mid-veins, 5 sent up flowering side branches with foliage and bracts similar to *Lamarckiana*, but all of the plants were small-flowered (petals about 2 cm long), as in culture 14.51.

HYBRIDS OF THE CROSS *BIENNIS* × *FRANCISCANA* B IN THE SECOND GENERATION, FROM SEEDS GERMINATED IN THE EARTH

A culture (14.56) was grown from seeds of the F_1 hybrid plant 13.36 *a*, *biennis* × *Franciscana* B (figure 10), described in my earlier paper (DAVIS 1914, pp. 190, 191). The sowing was made in earth, seed-pans being kept for 8—10 weeks, and the germination was very poor since from 637 seed-like structures (contents of 4 capsules) only 54 seedlings appeared, a germination of 8 percent. That this unsatisfactory result was due to delayed germination has since been established by experimental germination of more seeds from the same parent plant, 13.36 *a*, when 438 seedlings (culture 15.52) resulted from 623 seed-like structures, a germination of 70 percent.

Of the 54 seedlings, 51 developed rosettes which sent up flowering shoots during the summer. Since this culture was but a small fraction of the possibilities in the seeds sown, it obviously has no genetical value, and I shall only record its general character and the presence of one remarkable variant.

The culture was very uniform in the habit and foliage of the plants which resembled closely the parent F_1 hybrid 13.36 *a* (figure 10). They were large plants with narrow leaves of the *Franciscana* form and texture but larger. The pubescence was also like that of *Franciscana*, all plants having red papillae on the stems and ovaries. The buds had the thick sepal tips and pubescence of *Franciscana*, but the flower size varied from petals 1.1 to 2.8 cm in length. Most of the flowers were very close to the form and size presented in the F_1 parent hybrid.

A remarkable variant, 14.56 *w*, appeared with all flowers having cream-white petals (2.8 cm long); in other respects it resembled the other plants of the culture. This striking modification of color seems to parallel the change which takes place when the color variety *sulfurea* is thrown off from the Dutch *Oenothera biennis* as described by STOMPS (1914) and DE VRIES (1915 a). That the *biennis* parent of my cross should also have come from Dutch lines adds to the interest in this remarkable plant. The plant when selfed set an abundance of seed and should prove an important form for future study.

DISCUSSION AND SUMMARY

The chief interest in the results here presented lies in the clear evidence that an F_2 generation of a species cross of Oenothera may produce several classes sharply distinguished from one another and without inter-

grading types. The studies have an especial value since the germination of seeds in some of the cultures was experimentally determined to be complete. They are the first cultures of this character to be described and although the mortality among the seedlings was great it seems probable that the cultures were much more largely representative of their genetical possibilities than when in past years seeds have been sown in the earth.

This behavior, i.e., the production of classes in the progeny of hybrid oenotheras, has been described in the writings of several students of Oenothera genetics. Thus the twin and triple hybrids discovered by

FIGURE 26.—Inflorescence from the plant 14.55 *ac*, one of the most beautiful of the F_2 hybrids from *Franciscana* B × *biennis*. *Lamarckiana*-like as to flowers and foliage and as a rosette (figure 24), but with inflorescence less compact and buds more slender than in *Lamarckiana*. At the right is a leaf from the large rosette.

DE VRIES 1907, 1909), although appearing as the products of direct crosses, may in reality be of generations later than an F_1 if either or both of the parents were hybrid in constitution. The studies of the author on hybrids between *grandiflora* and a form of "American *biennis*" (DAVIS 1912, 1913) clearly indicated the differentiation of classes in the F_2 as well as phenomena apparently parallel to the production of twin hybrids in certain F_1 generations, the latter situation indicating that the *"biennis"* parent type was impure. The observations of ATKINSON (1914) on twin and triple hybrids of *Oenothera nutans* and *Oe. pycnocarpa* seem to furnish another illustration of segregation following a direct cross between parents of doubtful genetic purity. The interesting correlations of pigment distribution reported by SHULL (1914) in crosses of *rubricalyx* with *Lamarckiana* and *rubrinervis* likewise suggest segregation of characters from complex material. And the studies of GATES (1914) on hybrids between *grandiflora* and *rubricalyx* seem to the writer inconclusive for his contention that the behavior is non-Mendelian, when it is recognized how incomplete may have been the representation of the genetical possibilities in his frequently small and earth-sown cultures. The uncertainty of complete representations or of correct ratios which attaches to all of this earlier work, because of the failure to allow for the probabilities of incomplete germination and for the factors of gametic and zygotic sterility, have been sufficiently emphasized in the introduction.

The most striking of my results were exhibited by the F_2 of the cross *Franciscana* E $\times$ *biennis* in cultures where seed-germination was complete. If table 1 be examined with reference to a number of characters several interesting correlations which have the appearance of linkage, will become evident. (1) There was a large group of plants (182) *Franciscana*-like as to rosettes, stem coloration, foliage, bud tips and capsules, and a smaller group (16) similar to this but with narrower leaves. (2) A large group (67) *biennis*-like in rosettes, foliage, bud tips and capsules had, however, the stem coloration of *Franciscana*. (3) Green stems as in *biennis* were found only in a group (24) with rosette leaves obtuse-pointed and with foliage *Franciscana*-like but broader-leaved, and with buds and capsules also *Franciscana*-like. (4) A group (35) with evanescent rosettes contained a large number of plants (29) *Franciscana*-like and a small number (4) *biennis*-like in foliage, bud tips and capsules, while all of the plants had the stem coloration of *Franciscana*. (5) The dwarfs (59) with rosette leaves sickle-shaped were likewise mostly *Franciscana*-like (36), but some resembled *biennis* (3) in

foliage, bud tips and capsules although all agreed in having the stem coloration of *Franciscana*. This seems to me evidence clear-cut for the presence of distinct classes in the culture and I can see no reasonable interpretation except on the basis of a process of segregation determining different types of gametes at the end of the first hybrid generation, a behavior Mendelian in its fundamental nature.

The distribution of floral characters with reference to the classes which as to foliage were either *Franciscana*-like or *biennis*-like is given in tables 2 and 3. There is clearly a tendency to link within the same flower large petals or petals intermediate in size with a high position of the stigma, and conversely small petals were generally associated with a low or intermediate position of the stigma. However, while most of the *Franciscana*-like plants had large flowers and most of the *biennis*-like plants small flowers, there were numerous exceptions to the rule and no fixed association was discovered. It will be remembered that DE VRIES (1911, 1913) reported similar ranges of variation in flower size in hybrids patroclinous or matroclinous with respect to vegetative characters.

A remarkable feature of this cross (*Franciscana* E × *biennis*) in the F_2 was its clear segregation with respect to foliage into two groups, one resembling the *Franciscana* parent (274 plants) and the other the *biennis* type (70 plants). All of the data obtained on bud tips and capsules (see table 1) indicate that these characters of the parents also followed in their segregation the behavior of the foliage. The situation thus indicates that a large group of characters are closely correlated and their factors in inheritance are apparently closely linked. It is interesting to note that the differences in foliage characters bear a marked resemblance to the peculiarities that separate the twin hybrids, *laeta* and *velutina*, discovered by DE VRIES (1907) when *Lamarckiana* and certain of its "mutants" were crossed with *biennis* and some other forms. The hypothesis of RENNER (1914) that *Lamarckiana* is a heterozygote of a cross involving *laeta* and *velutina* forms takes on added interest from the behavior of the F_1 hybrids of *Franciscana* × *biennis*, for these species with respect to foliage characters have respectively the *laeta* and *velutina* characteristics. I shall cross certain *Lamarckiana*-like hybrids of *Franciscana* B × *biennis* with the Dutch *biennis* and with DE VRIES's "*biennis* Chicago" to determine whether they will give a twin progeny.

Among the *Franciscana*-like plants were a large number (about 52), which in all of their characters so closely resembled the *Franciscana* parent as to be virtually indistinguishable by taxonomic tests. Among

the plants *biennis*-like in foliage were 33 individuals with rosettes, bud tips, flowers, and capsules also as in the *biennis* parent, but none of these had green stems and ovaries (without red papillae) and consequently there were no segregates that completely matched the *biennis* type. Apparently factors for green stems either fail to link during segregation with other *biennis* factors, or if sometimes they do so segregate, the zygotes are not fertile. At least in a fairly large culture all of the 23 green-stemmed plants had foliage characters of *Franciscana*. There is indicated by these facts a high degree of correlation among the characters and it seems probable that much of the inheritance in oenotheras will exhibit extensive linkage of factors such as is known in other groups.

The F_2 from the reciprocal cross, *biennis* $\times$ *Franciscana* E, also from seeds completely germinated, gave a smaller number of classes than in the F_2 from the cross *Franciscana* E $\times$ *biennis*, but there was present a similar correlation of characters indicating extensive linkage of factors. As shown in table 4 the culture consisted of (1) a large group (186) *Franciscana*-like in rosettes, stem coloration, foliage, bud tips and capsules together with a small group (17) similar except for narrower leaves; (2) a group intermediate between the parents as to rosette characters but *Franciscana*-like in other respects; and (3) a group of dwarfs with sickle-shaped rosette leaves but also *Franciscana*-like in other characters. There were thus in the culture no classes *biennis*-like in rosettes, stem coloration, foliage, bud tips or capsules, and the only conspicuous suggestions of this parent appeared in rosettes intermediate in character and in the flowers more frequently small and with a low position of the stigma. The culture was therefore distinctly one-sided and *Franciscana*-like in the expression of its vegetative characters but the high degree of sterility, gametic and zygotic, may account for this fact through the elimination of types which theoretically would be expected as the result of a normal segregation from such a cross. On the assumption that forms *biennis*-like in various respects were thus eliminated the surviving groups may be held to represent one side (*velutina*) of a segregation theoretically probable. The seed-fertility of only 57 percent, the extensive pollen-sterility, and the high mortality in the culture are ample to allow of such a possibility.

It is curious that this culture, generally *Franciscana*-like in vegetative expression, should for the most part have had small flowers or flowers in size intermediate between the parents, yet an examination of tables 5 and 6 will show this to have been the case. With the small flowers was generally associated a low position of the stigma. However, 6

plants, *Franciscana*-like in vegetative characters, also had large flowers with a high position of the stigma and these plants were essentially indistinguishable from the *Franciscana* parent.

Neither of the two cultures presented flowers appreciably larger than those of the *Franciscana* parent, but there were 15 plants in the F_2 from *Franciscana* E × *biennis* and 7 in the F_2 from the reciprocal the flowers of which were smaller than the *biennis* type. The petals of these plants were from 9—15 mm long and generally crumpled; the plants in other respects seemed normal and set seed abundantly. Future cultures will determine whether or not this character is germinal.

There are also described in this paper hybrids in the F_2 from a cross between another line of *Franciscana*, designated as *Franciscana* B, and the same race of *biennis*. The cultures, grown in 1914, were from seed germinated in the earth and undoubtedly only imperfectly indicated the genetical possibilities of the cross, but they, nevertheless, presented a greater variety of types in the F_2 than were found in the crosses between *Franciscana* E and *biennis*. The indications are clear that *Franciscana* B in spite of its taxonomic resemblance to *Franciscana* E has not in reality quite the same germinal constitution.

Cultures in the F_2 of *Franciscana* B × *biennis* were grown from seeds of 5 sister F_1 plants which differed in relatively small particulars and each of the 5 families from these F_1 parent plants exhibited its own characteristics. Consequently the F_1 generation was clearly made up of somewhat diverse biotypes, a condition not uncommon in species crosses and which I also found in my studies on hybrids between *grandiflora* and an "American *biennis*" (DAVIS 1912, 1913).

The most interesting forms in the F_2 from the cross *Franciscana* B × *biennis* were the following: (1) A number of plants appeared very similar to *Lamarckiana* in vegetative characters and some of these bore large flowers. One individual, 14.53 *c*, with large flowers and short capsules has been selected as the starting-point of a line already taken through the F_3 in the expectation that a *Lamarckiana*-like hybrid may be synthesized. (2) Probable triploid types (chromosomes counted up to 19 and 20), comparable in their size and self-sterility to the *semigigas* derived from *Lamarckiana* and *biennis*, were represented by 3 plants which appeared each in a different family. (3) A single plant, 14.51 *r*, with a foliage of broad leaves bore flowers in most cases with style and stigma withered to a brown filament rendering them infertile, a condition suggestive of the chief peculiarity of *brevistylis*. (4) A large number of dwarfs with acute-pointed, sickle-shaped rosette leaves

corresponded to a similar group in the F_2 from the cross *Franciscana* E × *biennis*. (5) Etiolated dwarfs similar to DE VRIES's *albida* were represented by 4 rosettes which died early, and there were also 3 rosettes with green leaves almost linear, which resembled *sublinearis* DE VRIES.

The mass of the F_2 cultures consisted of two groups distinguished as rosettes. The first of these had rosette leaves broad and obtuse-pointed, a foliage of broad leaves, and small flowers of the *biennis* type except that the bud tips were *Franciscana*-like; these plants constituted from 32—41 percent of the cultures. The second group was less uniform but had rosette and foliage leaves narrow and acute-pointed, flowers that in form and size ranged between those of the parents, and sepal tips in some plants thick as in *Franciscana* and in others long and slender as in *biennis* and *Lamarckiana*. There was probably material here for the differentiation of a number of classes but my data is too imperfect to discuss this point.

A number of plants were noted taxonomically indistinguishable from the *Franciscana* parent, but none that had all of the characters of *biennis*. Segregates of the *biennis* type were absent by virtue of the fact that all of the plants in the culture had the stem coloration (red papillae) of the *Franciscana* parent. That a class of green-stemmed plants was represented in the zygotes seems to be established by another culture grown a year later (1915) from sister seeds germinated experimentally. In this culture a group of 14 green-stemmed, small-flowered plants appeared, but this culture, started late in the season, failed to send up many shoots and the data from it has little genetical value.

The dwarfs, distinguished as rosettes by their sickle-shaped leaves, which appeared in these F_2 cultures as well as those from the crosses between *Franciscana* E and *biennis* deserve brief discussion. There was much variation in the extent of the asymmetrical development shown by the leaves, a character which has been reported in other cultures of Oenothera (see GATES 1913, pl. I, fig. 12, *Oe. Lamarckiana* var. *cruciata*), and is not infrequently shown to a greater or less degree in the rosettes of *Oenothera nanella* DE VRIES. I have noted that occasionally from such rosettes full-sized normal plants may develop instead of the stunted irregular growths generally present. This opens the question whether or not the sickle-leaved dwarfs really constitute a class distinguished by a particular genotype or whether their peculiarities may not be due to a diseased condition. Further genetical studies will be necessary to determine this point.

The F_2 generation from the reciprocal cross *biennis* × *Franciscana* E

was represented by a very small culture due to delayed germination. It was chiefly interesting for the appearance of a plant with cream-colored petals, a color variety which apparently parallels the form *sulfurea* thrown by *biennis*.

In conclusion, the results of these studies on hybrids between *Oenothera biennis* and *Oe. Franciscana* appear to the writer to give positive evidence of a segregation of factors in the F_2 generation of a character to be expected in Mendelian inheritance. The differentiation of the classes in the F_2 was unmistakable and a remarkable feature of these classes was the firm correlation of many characters indicating a remarkable linkage of certain factors. That the situation, if Mendelian, is very complex becomes apparent from the extent of the linkage. Although certain of the cultures, because grown from seed forced to a complete germination, are probably fairly representative of the genetical possibilities of the F_1 hybrid parents it must be remembered that very large numbers of plants either died or could not be brought to maturity. Furthermore, there was a large amount of sterility in the pollen (about 50 percent) and to almost as great a degree in the seeds produced by the F_1 generation. For these reasons the author has refrained from speculation on the possible ratios that might be suggested for the appearance of classes or of characters on Mendelian hypotheses. The high degrees of sterility both gametic and zygotic present problems of irregularities so great as to render speculation at present most unsafe. Also, the parent species have not themselves passed the tests of a pure species (DAVIS 1915 b), and there are suggestions in the facts of seed-sterility in *Franciscana* and of pollen-sterility in *biennis* that these species are not strictly homozygous. Mendelian studies on the oenotheras seem to the writer at present to be quite hopeless except as they are concerned with the simplest conditions that can be experimentally devised and deal with parent material exhaustively tested for its genetic purity or of which the genotypes are most thoroughly known.

University of Pennsylvania
December 1915

LITERATURE CITED

ATKINSON, G. F., 1914 Segregation of unit characters in the zygote of Oenothera with twin and triplet hybrids in the first generation. Science **39**: 834-835.

BARTLETT, H. H., 1913 The delimitation of *Oenothera biennis* L. Rhodora **15**: 48-53.

1914 *Oe. Franciscana* and *Oe. venusta* spp. novo. Rhodora **16**: 33-37.

1915 a Additional evidence of mutation in Oenothera. Bot. Gaz. **59**: 81-123.

1915 b The mutations of *Oenothera stenomeres*. Amer. Jour. Bot. **2**: 100-109.

1915 c Mutations *en masse*. Amer. Nat. **49**: 129-139.

1915 d Mass mutation in *Oenothera pratincola*. Bot. Gaz. **60**: 425-456.

DAVIS, B. M., 1912 Further hybrids of *Oenothera biennis* and *Oe. grandiflora* that resemble *Oe. Lamarckiana*. Amer. Nat. **46**: 377-427.

1913 a The behavior of hybrids between *Oenothera biennis* and *Oe. grandiflora* in the second and third generations. Amer. Nat. **47**: 449-476, 547-571.

1913 b The problem of the origin of *Oenothera Lamarckiana*. New Phytol. **12**: 233-241.

1914 Some reciprocal crosses of Oenothera. Ztschr. f. ind. Abst. u. Vererb. **12**: 169-205.

1915 a A method of obtaining complete germination of seeds in Oenothera and of recording the residue of sterile seed-like structures. Proc. Nat. Acad. Sci. **1**: 360-363.

1915 b The test of a pure species of Oenothera. Proc. Amer. Phil. Soc. **54** 226-245.

1915 c Additional evidence of mutation in Oenothera? Amer. Nat. **49**: 702-706.

DE VRIES, H., 1907 On twin hybrids. Bot. Gaz. **44**: 401-407.

1909 On triple hybrids. Bot. Gaz. **47**: 1-8.

1911 Ueber doppeltreziproke Bastarde von *Oenothera biennis* L. und *Oenothera muricata* L. Biol. Centralbl. **31**: 97-104.

1913 Gruppenweise Artbildung. pp. viii + 365. Berlin: Gebrüder Borntraeger.

1915 a The coefficient of mutation in *Oenothera biennis* L. Bot. Gaz. **59**: 169-196.

1915 b Ueber künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. **35**: 161-176.

1915 c *Oenothera gigas nanella*, a Mendelian mutant. Bot. Gaz. **60**: 337-345.

EAST, E. M., 1912 The Mendelian notation as a description of physiological facts. Amer. Nat. **46**: 633-655.

GATES, R. R., 1913 A contribution to a knowledge of the mutating oenotheras. Trans. Linn. Soc. Botany **8**: 1-67.

1914 Breeding experiments which show that hybridization and mutation are independent phenomena. Ztschr. ind. Abst. u. Vererb. **11**: 209-279.

1915 a The mutation factor in evolution with particular reference to Oenothera. pp. xiv + 353. London: Macmillan and Co.

1915 b On successive duplicate mutations. Biol. Bull. **29**: 204-220.

HERIBERT-NILSSON, N., 1912 Die Variabilität der *Oenothera Lamarckiana* und das Problem der Mutation. Ztschr. ind. Abst. u. Vererb. **8**: 89-232.

1915 Die Spaltungserscheinungen der *Oenothera Lamarckiana*. Lunds Universitets Arsskrift N. F. **12**: 1-132.

LUTZ, ANNE M., 1912 Triploid mutants in Oenothera. Biol. Centralbl. **32**: 385-435.

RENNER, O., 1914 Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. Flora **107**: 115-150.

SHULL, G. H., 1914 A peculiar negative correlation in Oenothera hybrids. Jour. Gen. **4**: 83-102.

STOMPS, T. J., 1912 Die Entstehung von *Oenothera gigas* de Vries. Ber. deutsch. bot. Gesell. **80**: 406-416.

1914 Parallele Mutationen bei *Oenothera biennis* L. Ber. deutsch. bot. Gesell. **32**: 179-188.

STUDIES ON OAT BREEDING. III. ON THE INHERITANCE OF CERTAIN GLUME CHARACTERS IN THE CROSS *AVENA FATUA* × *A. SATIVA* VAR. KHERSON[1]

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INTRODUCTION

In 1912 some work was undertaken on the behavior of certain characters in crosses between various species and varieties of Avena. In that year a number of crosses were made involving among other things a wild *Avena fatua* and various cultivated varieties of *Avena sativa*. It is the purpose of this paper to discuss the behavior of certain glume characters in one of these crosses, viz., wild and Kherson. The original crosses discussed here were made by Dr. MAYNIE R. CURTIS of this laboratory, and the writer desires to express his appreciation of her kindness in turning this and other material over to him in the spring of 1913.

The present paper deals only with the inheritance of certain qualitative

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 95.

characters of the flowering glumes[2] of these two varieties. Data have also been collected relative to a large number of other characters, particularly quantitative characters. The analysis of these will be published in a later paper.

A word should be said regarding the methods used. All crosses were made in the field. After emasculation the heads were kept bagged until harvested. All the plants concerned in the present discussion were grown out-of-doors. The method of handling the mature plants has differed slightly in different years. In some years the plants have been pulled, the heads covered with large paper bags and stored in a grain room. Later each plant was examined, the required data recorded and the grain threshed out. In other years various data regarding the height, type of head, etc., have been recorded in the field and the heads of each plant placed in a separate labelled envelope. Data regarding grain characters, etc., were recorded during the winter.

All of the data from the F_2 and F_3 plants have been recorded by punching specially prepared cards adapted for use in an automatic card sorting machine. Quantitative characters are punched directly on the cards, while with qualitative characters it is necessary to arrange an arbitrary code for each character.

A large amount of the actual recording of the data and the punching of the cards has been done by Mr. W. E. CURTIS and Mr. C. H. WHITE. The writer desires to express his appreciation of the carefulness of this work.

THE WILD PARENT (*AVENA FATUA*)

The wild parent used in these crosses was secured as a stray plant selected from a field in 1910. Seed from this plant was sown in 1911 and bred true in respect to all of its characters. Since then it has been grown every year as a pure line known in our records as line No. 274. Of the several hundred plants of this line that have come under observation, none has shown characters different from those described below. This oat undoubtedly belongs to the *Avena fatua* group, but inasmuch as

[2] As is well known, in all species of oats, except the naked *A. nuda*, the flowering glumes or bracts adhere firmly to the grain proper or caryopsis. There is present also on each spikelet a pair of outer empty glumes. In order to avoid the necessity of specifying "flowering" glumes each time they are referred to, the word "grain" in the following paper will frequently be used to designate the *Caryopsis enclosed in its flowering glumes*. In case reference is made to the grain proper, the term caryopsis will be used. With this explanation there should be no confusion in the use of these terms.

we have obtained several quite different races, all probably belonging to this group, it seems necessary to describe this particular pure line in some detail.

The plants grow very tall, averaging about 195 centimeters (6½ feet) when grown in garden rows. They show a relatively large amount of stooling. When given ample space they frequently develop from 8 to 15 culms. In the younger stages of growth the culms tend to spread out over the ground showing a recumbent habit quite different from that of most cultivated races. After the heads begin to form the plant loses this appearance. The straw is medium weak and unless supported is almost sure to break down before the grain is ripe. Unlike the cultivated forms of oats, the grain ripens very unevenly. It is not unusual to find some heads ripe and the grain shattering, while other heads on the same plant are still blooming.

The heads are very long, averaging about 36.5 centimeters. Figure 1 (plate 2) shows the character of the heads. The branches are long and spreading with a tendency to droop. The distance between the whorls is much greater than in the common varieties of cultivated oats.

Figures 2, 3 and 6 (plates 2 and 3) show the glume characters of this oat.[3] In color it is a dark reddish brown. The callus or base of each grain is expanded into a sucker-like ring (figure 3). This large cleavage plane permits the grain to separate from the outer glumes very easily when mature. The grain shatters so easily that it is often necessary to bag the heads in the field in order to secure enough mature grain for the next season. As shown in figure 2, both the upper and lower grains in each spikelet bear strong awns. The basal portion of each awn is dark-colored and twisted, while the distal portion is lighter-colored and bent at nearly a right-angle to the basal part (geniculate).

Various portions of the glumes of both grains are covered with a stiff pubescence. Attention will be directed to the following areas in this paper. (1) The callus ring at the base of the grain is covered on its

[3] A word should be said regarding the terms used in describing oat grains. As is well known, a spikelet of common oats usually bears two grains. One of these is much larger than the other and is called the "lower" grain. The smaller or "upper" grain is articulated with the lower by means of a short pedicel (figure 7). In case a "third" grain is present (figure 4) this is borne on a pedicel attached to the upper grain. In the vast majority of spikelets this third grain is represented by an aborted flower. The pedicel, however, is always present on the upper grain. The area of attachment of either grain is known as the callus. In this paper this whole region will most often be referred to as the "base of the grain." The side of the grain which bears the awn, if one is present (figure 2) will be called the dorsal, and the opposite the ventral. The "sides" of the grain will then be the lateral sides.

lateral and dorsal sides with a thick growth of stiff, rather short hair. This is true of both the upper and the lower grains. (2) The back of each grain, upper and lower, is thickly covered with a rather long pubescence. (3) The pedicel on the lower grain, which bears the upper, is also covered with a thick long pubescence (figure 3). The same is true of the pedicel on the upper grain, although usually this bears only an aborted grain. In all cases the pubescence has a brownish color.

THE CULTIVATED PARENT (KHERSON)

The wild parent described above has been crossed with a number of cultivated races but since only one of these crosses is discussed in the present paper, the following description will be restricted to it. The cultivated race used in this work is a pure line, selected from the Kherson variety in 1910. This pure line is known in our records as line No. 26. It has now been grown for 5 years and has always bred true to all of its characters.

The Kherson variety is one of the early or so-called 60-day varieties of oats. With us, in Maine, it requires from 90 to 95 days to mature, about 10 days less than the ordinary medium oat. When grown under the same conditions as the wild referred to above, this oat attains an average height of about 130 centimeters. It possesses a small but rather stiff straw. As shown in figure 5 the head is short, the average length being less than 20 centimeters. The branches are short and have a tendency to stand at right angles to the main axis, or even to droop somewhat. The drooping is not so pronounced as in the wild.

The grain characters of this oat are shown in figures 4 and 7. In color the grain is a well defined yellow.

Awns are entirely absent or occasionally there may be a very weak awn on the back of the lower grain. The callus or base of the grain shows a small notched articulation quite different from that of the wild grain. (Cf. figures 3 and 7.) In the following paper this base is called the "cultivated" as contrasted with the "wild". There is no pubescence on the back or on the pedicel of either grain or on the base of the upper grain. Ordinarily there is no pubescence at the base of the lower grain, but on a few grains two or three rather long hairs may be found at the sides of the callus. These hairs are very easily broken off and can only be observed on grain freshly removed from the outer glumes. Even then only a small percent of the grains show these hairs. All of the cultivated varieties of oats which have come under the writer's notice may have this

PLATE 2

FIGURE 1.—Photograph of a head of wild oats, *Avena fatua*, line no. 274. × ¼.

FIGURE 2.—Grains from a spikelet of wild oats showing the awns and pubescence on both grains. × 2.

FIGURE 3.—Ventral surface of the lower grain from spikelet of wild oats showing the wild base, the pubescence on the pedicel and the sides of the grain. × 8.

FIGURE 4.—Grains from a spikelet of Kherson oats showing the complete absence of awns and pubscence. × 2.

FIGURE 5.—Photograph of a head of Kherson oats, line no, 26. × ¼.

PLATE 3

FIGURE 6.—Dorsal surface of the upper grain from a spikelet of wild oats showing the awn and the pubescence on the back and base. × 8.

FIGURE 7.—Ventral view of the lower grain from a spikelet of Kherson oats showing the cultivated base of the grain and the absence of pubescence. × 8.

FIGURE 8.—Grains from F_1 plants, wild × Kherson. Lower grain pubescent on the back, the upper grain smooth. Lower grain sometimes has a weak to medium strong awn. × 2.

FIGURE 9.—Ventral view of the lower grain from an F_1 plant, showing the intermediate base, pubescence at sides of the base and absence of pubescence on the pedicel. × 8.

FIGURE 10.—Wild × Kherson F_1. A. Dorsal view of lower grain. B. Dorsal and ventral view of upper grain. × 2.

FIGURE 11.—Wild × Kherson F_2 — cultivated black grain pubescent on the back of the lower grain but the upper grain is smooth. × 2.

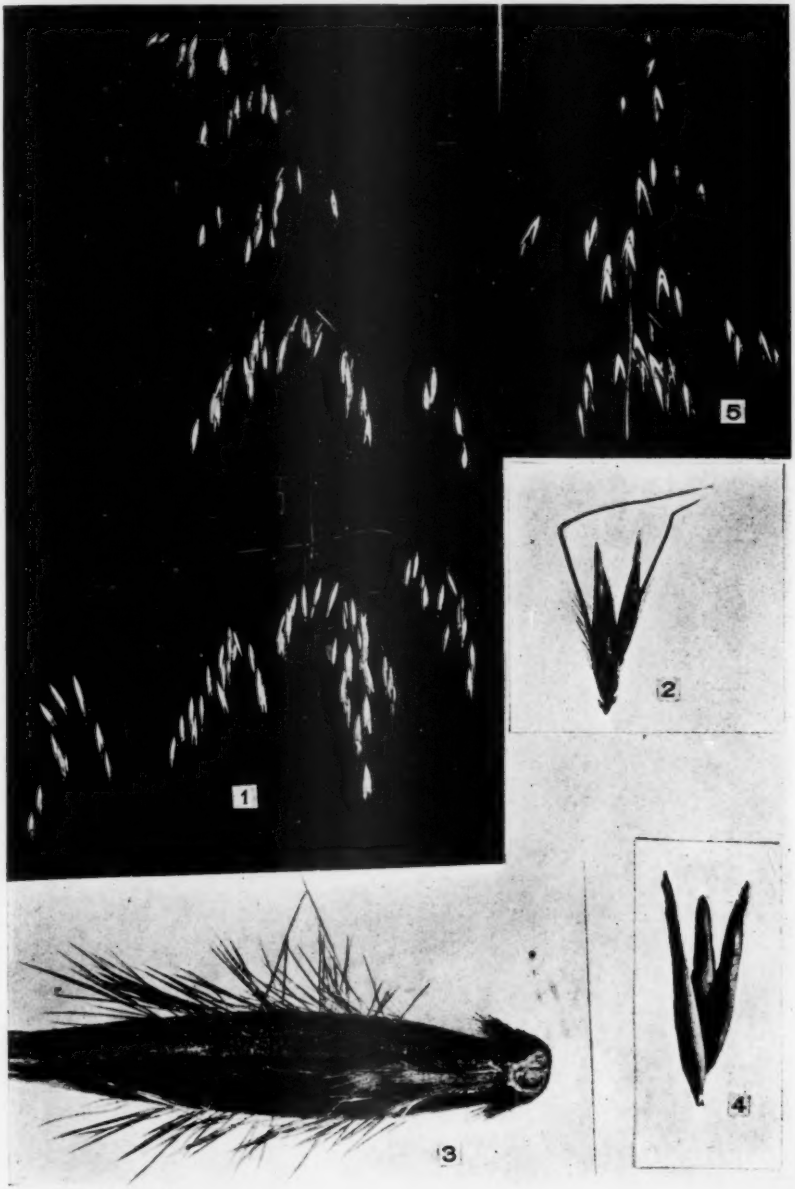
FIGURE 12.—Wild × Kherson F_2 — wild gray, showing absence of pubescence on the back of either grain. × 2.

FIGURE 13.—Wild × Kherson F_2 — wild yellow, showing the absence of pubescence on either grain. × 2.

Figure 14.—Wild × Kherson F_2 — cultivated black grain showing no pubescence on the back of either grain. Only two plants of this kind appeared in the F_2 generation. × 8.

FIGURE 15.—Wild × Senator F_2 — White grain with pubescence on the back of each grain. A. Dorsal view of upper grain. No representatives of this class appeared in the F_2 generation of wild × Kherson. × 2.

FIGURE 16.—Wild × Kherson F_2 — cultivated black grain with pubescence on the back of each grain. Only two plants of this kind appeared in the F_2 generation. × 8.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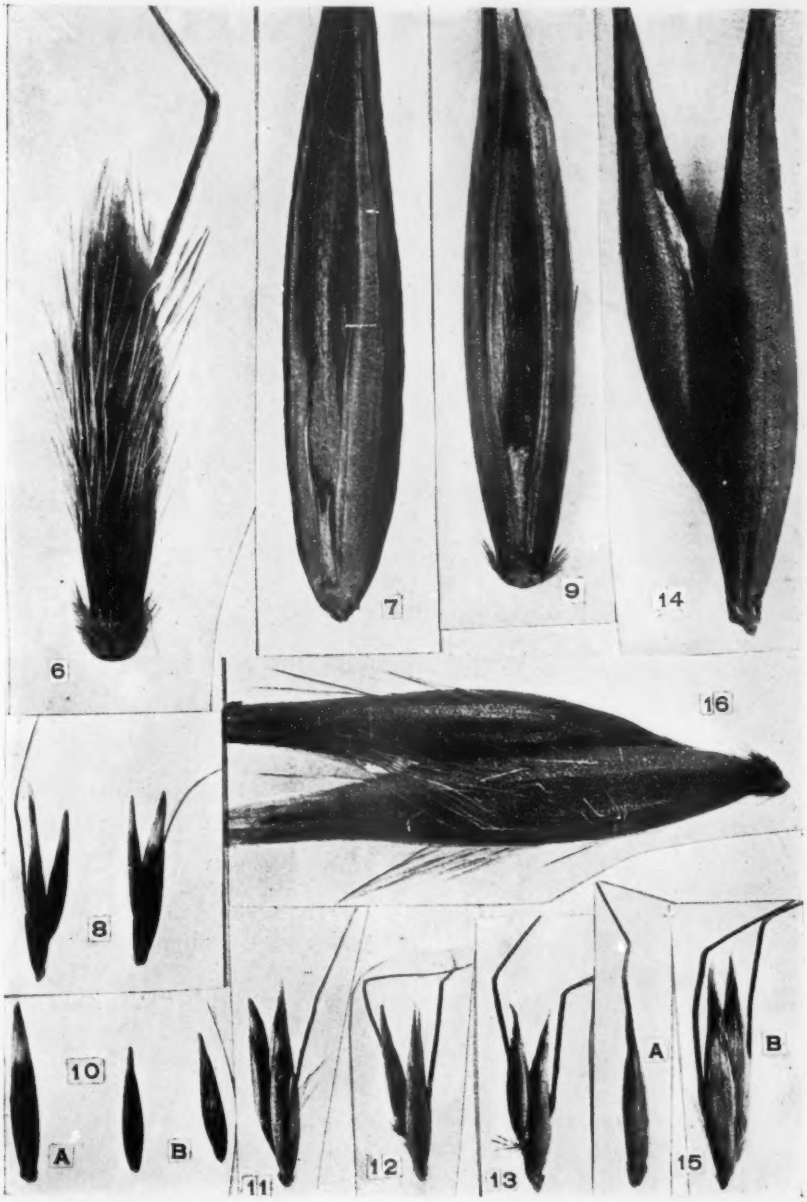
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slight pubescence at the base of the lower grain. Some varieties have it more marked than others.

Table 1 gives a list of some of the contrasting characters of these two races of oats.

TABLE 1

Character	Wild Line No. 274	Kherson Line No. 26
Height of plant	195 cm	130 cm
Length of head	36.5 cm	19.8 cm
Width of head	23.1 cm	12.1 cm
Diameter of straw	.33 cm	.25 cm
Type of head	Wide-spreading, drooping	Spreading
Grain color	Dark brown	Yellow
Base of grain	Wild type	Cultivated type
Shattering	Shatters	Does not shatter
Awns	Heavy, twisted and geniculate awns on both upper and lower grains.	None or very few on the lower grain. None on the upper.
Pubescence—		
Base of grain	Thick pubescence on lateral and dorsal sides of callus on both grains.	None or occasionally 1 or 2 hairs at the sides of the base of the lower grain.
Back of grain	Heavy pubscence on the back of both grains.	None
Pedicel	Heavy pubescence on both grains.	None

THE F_1 GENERATION

Fifteen successful crosses between these two varieties were made in 1912. The F_1 plants were grown out of doors in 1913. One of the fifteen seeds failed to germinate. The fourteen F_1 plants belonged to six different pedigrees in the sense that they came from six different combinations of male and female plants. The following five pedigrees were from crosses in which the wild was the male parent: Pedigree No. 551, 2 plants; No. 553, 1 plant; No. 554, 1 plant; No. 558, 6 plants; and No. 559, 1 plant. Pedigree No. 609, containing three plants, was the reciprocal cross, with the wild as the female parent. So far as the characters discussed in this paper are concerned no differences in either F_1 or F_2 occurred in the reciprocal crosses.

The fourteen F_1 plants were remarkably uniform in all their characters. In the majority of their characters the F_1 plants were inter-

mediate between the two parents. Thus the average height was 148 centimeters. Our notes record the heads as long, wide-spreading, although the actual measurements show that they were almost exactly intermediate. The branches stood at nearly right-angles to the stem and showed less tendency to droop than in the wild.

The color of the grain is a light reddish brown, distinctly lighter than the wild parent. There is some variation in the color of different grains. To some extent this variation is dependent upon the state of maturity of the individual grains at the time of harvest. Quite often the grain in some of the later culms is not mature at the time of harvest. In any black or brown oat such grains are always lighter in color. Aside from this, however, there are distinct variations in color. A few grains contain as much pigment as the original wild. The majority are intermediate. In the ordinary Mendelian use of the word, however, the brown or black color is dominant. No grains approach the yellow color of the Kherson.[4]

Figure 9 shows a ventral view of the lower grain of a spikelet from an F_1 plant. By comparison with figures 3 and 7 it will be seen that the base of the grain is unlike either the wild or cultivated parent. It is in fact distinctly intermediate, showing a much larger articulation than the cultivated grain, yet resembling it more than the wild. This F_1 grain does not shatter like the wild and hence resembles the cultivated parent. In general terms it may be said that the cultivated base is dominant.

The base of the upper grain as shown in figure 8 is very similar to the condition in the cultivated race.

No awns are ever found on the upper grain but the majority of the lower grains show a weak, straight awn without a distinct basal portion (figure 8). Other spikelets lack any trace of awns. This condition is again intermediate between the two parents, for the Kherson may be said to lack awns entirely. The conditions in these F_1 plants is quite similar to that found in many cultivated varieties.

Figures 8, 9 and 10 show that the lower grain of the F_1 plants possesses a tuft of hair at either side of the base. Unlike the wild parent this pubescence does not extend over the back of the callus. It has been stated that occasionally cultivated oats show one or two hairs at the sides of the base. In these F_1 plants this condition is greatly augmented,

[4] It should perhaps be noted that in any black or brown oat a grain in which the caryopsis has failed to develop will not show the brown color. Thus in any threshed grain of this kind some grains may appear almost white. Upon examination it will be found that these contain no kernel.

so that in every spikelet the lower grain has a rather thick tuft of hairs at the sides of the base. There is no pubescence at the base of the upper grain.

There is no pubescence on the pedicel on either grain. Occasionally a few very short hairs will be found on the pedicel of the lower grain but this character is so variable in grain from the same head and occurs so rarely that no significance has been attached to it. In fact quite similar conditions can be found in the grain of some cultivated varieties.

The back of the lower grain shows a medium pubescence (figures 8 and 10). This pubescence is very much shorter and thinner than that on the wild parent. In the majority of cases it is confined to the sides of the grain. On other grains it extends over the entire back as in the wild. In general, this character is intermediate between the two parents. From the standpoint of the presence and absence of hairs, this pubescence on the back of the lower grain is dominant.

The back of the upper grain is entirely free from pubescence in all cases (figure 10). The smooth condition of the cultivated grain is completely dominant.

THE F_2 AND F_3 GENERATIONS

Some F_2 plants were grown from each of the fourteen F_1 plants. The majority of these plants were grown in 1914 but some additional plants were grown in 1915. The plants grown in the two years show the same segregation in all characters so far studied and hence are considered together. In all there are 465 F_2 plants upon which we have data.

A small F_3 generation was grown in 1915. Short rows containing 20 plants each, were planted from 75 different F_2 plants. The F_2 plants were in the main selected at random. Certain mishaps occurred during the season so that the number of plants harvested from some of the rows was much smaller than twenty. In the following discussion no rows are included which had less than ten mature plants unless it is specifically stated. The number of rows and the number of plants in each is too small to be of much significance. However, even this data frequently aids in determining the validity of a given hypothesis.

Color of the grain (glumes)

The wild parent has a dark brown or almost black color. The Kherson parent has a yellow color. In addition to this the F_2 segregation shows that the wild parent also carries a factor for gray color. There are thus three factors concerned: Brown or black (B), gray (G) and yellow

(*Y*). NILSSON-EHLE (1909) has discussed the expectation in such a cross. Several shades of brown, gray and yellowish gray, yellow and white are to be expected. It is almost impossible to separate the yellow and white in many cases. A white grain that has weathered slightly will have a yellowish tinge. Since these F_2 plants ripen rather unevenly, some plants have been over-ripe and slightly weathered when harvested. Further it is not possible to accurately separate the grays into gray and yellow gray. At least four distinct classes should be distinguished. On the three-factor hypothesis the relative proportions of these should be 48 black : 12 gray : 3 yellow : 1 white, the white of course being the triple recessive.

In classifying the F_2 generation only three plants were found which were apparently white, while the expectation is for 7.3 out of 465 individuals. On re-examining the grain from these three plants it was found that even they had a slight tinge of yellow but it could not be decided whether this was due to weathering or to the presence of a yellow color factor possibly in connection with a dilution factor. None of these plants was grown in the F_3 generation. It is however quite probable that the wild parent carries a yellow gene, in which case only three phenotypic color classes would be expected, viz., 12 black : 3 gray : 1 yellow. The present data are not sufficient to decide whether this is the correct hypothesis or not. It might be added that all the yellow F_2 plants (7) grown in the third generation bred true. The number of F_3 rows is too small to be decisive. In the following discussion the yellows and the possible whites will be grouped together.

Table 2 shows the observed and expected numbers obtained from the 465 F_2 plants.

TABLE 2

	Black	Gray	Yellow
Observed	347	88	30
Expected	*348.7*	*87.2*	*29.1*

It will be noted that the agreement between the observed and calculated numbers is exceptionally good.

In no case is there the slightest difficulty in classifying the blacks as contrasted with the non-blacks. There are, however, several different shades of black or brown and there is some evidence to indicate that these different shades are in part due to internal heritable genes. The

data in the present paper, however, are not sufficient to admit of a discussion of this question. It is hoped to take up this problem at a later time when the data from other crosses and from larger numbers of individuals are at hand.

The data from the F_3 generation relative to the blacks bear out the preceding hypothesis. Sixteen rows each containing ten or more plants bred true to black. Thirty-four rows showed splitting into blacks and non-blacks. Seven of these latter rows contained less than ten plants. The remaining twenty-seven rows contained from 10 to 25 plants each.

Table 3 gives the observed and expected number of rows on the assumption that the F_1 plants are homozygous for yellow. The agreement is only fair but the number of rows and the number of plants in each are too small to be of much value.

TABLE 3

Character of the F_3 Segregation	No. of F_3 rows	
	Observed	Expected
Black and gray	4	6.75
Black, gray and yellow	12	13.50
Black and yellow	11	6.75

Among the grays there are several shades, some of which are so light that it is difficult to distinguish them from yellows. NILSSON-EHLE (1909, p. 122) notes this difficulty and an examination of his ratios in cases where these factors are involved indicates that perhaps some of the plants classed by him as whites are in reality very light grays. I have found many plants which show no trace of the gray color on the dorsal side but which if examined on the ventral side show a distinct gray on the palea. Further such plants always show a darker gray on the upper grain and still darker on a third grain if that is present. In some cases the lower grain shows no trace of gray, while the upper shows it very distinctly, especially on the ventral side. When grown in the third generation such plants have invariably thrown some grays. They are, however, usually if not always heterozygous. The homozygous grays have a much more distinct color.

In taking the data on these plants the grays were recorded in two classes, dark grays and light grays. The numbers obtained in the F_2 generation were 43 dark to 45 light grays. This ratio does not agree with the expected numbers of homozygotes and heterozygotes. It is

possible that there are still other genes which may modify the intensity of the gray color.

In the third generation six of the dark gray plants were grown. Three of these bred true and three split into grays and yellows. The number is too small for the ratio to be significant perhaps but it shows clearly that some of the dark gray plants are heterozygous. Seed from eleven of the light gray plants were planted and every one split into grays and non-grays. From this it seems probable that the light grays are always heterozygous.

Seven F_2 yellow plants were grown in the third generation and all bred true to yellow. In these seven rows no plants appeared which could be classed as white.

These results as to the color of the grain are in excellent agreement with the assumption of NILSSON-EHLE (1909) that there are three separately inherited color factors, each allelomorphic to its absence. The F_1 plants are heterozygous for two of these factors, black and gray, but probably homozygous for the third, yellow.

In this connection it should not be forgotten that if one has a dislike for the presence and absence hypothesis the results can be equally well explained by assuming that each of these genes is allelomorphic to a white. Either hypothesis accounts for the observed results.

The base of the grain

As described above, the wild parent possesses a very broad sucker-like base (figure 3), which allows the grain to shatter very easily. On the wild parent this base is present on both the upper and lower grain of each spikelet. The same is true of a third grain if that is present. The cultivated races possess a narrow base (figure 7) which does not separate from the axis readily; under ordinary conditions this grain does not shatter. The F_1 generation presents an intermediate condition as regards the lower grain but it is more like the cultivated parent than the wild (figure 9). It does not shatter. The upper grain has a cultivated base similar to that of the upper grain on the cultivated variety.

The F_2 generation shows for the lower grain three distinguishable kinds of bases. These may be designated as the cultivated, intermediate and wild. The intermediate corresponds to that described for the F_1 plants (figure 9). There is some gradation between the cultivated and intermediate bases so that often it is difficult to classify them. If we assume that the difference between the cultivated and wild base is due to one pair of genes and that the intermediate base represents the simplex

condition, these three categories should occur in a 1 : 2 : 1 ratio. Table 4 gives the observed and expected results.

TABLE 4

Showing the segregation in regard to the base of the lower grain.

	Cultivated	Intermediate	Wild
Observed	117	236	112
Expected	*116.25*	*232.5*	*116.25*

It is clear that observation and theory agree very well in this case.

The intermediate base is much more nearly like the cultivated than the wild. In fact the two intergrade so that in some plants it is very difficult to decide upon the classification. The cultivated base may, therefore, be said to be dominant. For practical purposes in the further discussion of these results the plants showing the typical cultivated base and those with the intermediate base will be grouped together under the term "cultivated." The relation of certain characters to the intermediate condition will, however, be pointed out.

The cultivated base of the *upper* grain is completely dominant over the wild. No intermediates are found. There are no means in the present data to determine whether this latter character is determined by the same gene as the base of the lower grain or whether by a separate gene completely linked with the latter. In either case there is no somatic distinction between the simplex and duplex condition in regard to the dominant character in the upper grain.

In the F_3 generation 67 rows with 10 or more plants each were harvested. Fifty-three of these rows were from cultivated parents. Of these 18 bred true and 35 split into cultivated and wild plants. The 35 heterozygous rows gave 416 cultivated plants and 142 wilds. Fourteen F_3 rows from wild parents all bred true. These numbers are almost exactly in accord with expectation on the assumption that the difference between the cultivated and wild base is due to a single pair of genes.

a. Relation of the base to the color of the grain

The question as to whether the gene for the base of the lower grain segregates independently of the several color genes is of interest. Table 5 shows the distribution of the F_2 plants with regard to these characters. The cultivated and intermediate bases are grouped under cultivated (C).

TABLE 5

Showing the relation between grain color and the character of the base of the grain.

	Black		Gray		Yellow	
	C	W	C	W	C	W
Observed	259	88	73	15	21	9
Expected	*261.5*	*87.2*	*65.4*	*21.8*	*21.8*	*7.3*

From this table it is seen that the observed and expected numbers agree very well in the case of the black and the yellow. There is a slight discrepancy in the case of the grays. These are not beyond the possible fluctuation of random sampling. The standard deviation ($\sqrt{npq}$) of the expected number of wild grays is ± 4.56. The difference between the observed and expected is 6.8, or considerably less than twice the standard deviation.

There is little question but that the genes for the character of the base segregate independently of the color genes.

Characters correlated with the wild base

Reference has already been made to the fact that the wild base on the upper grain of a spikelet occurs only when the lower grain has a similar base. The same is true of a third grain when present. This correlation between the bases of the several grains on a spikelet may be regarded either as caused by the same gene or by two separate genes so completely linked that they are never found separated. In addition to this possible case of "linkage" there are several other characters which are also always associated with the wild base. There is no evidence as to which of the two hypotheses mentioned above more nearly accounts for the facts. All of the characters discussed in this section appear always to be associated together. In the F_2 and F_3 generations of this cross there is not a single exception to the associations discussed below.[6] For purposes of description these associations will be regarded as due to the linkage of several genes.

a. Awns

The first of these linked characters that will be described is the heavy awning found on the wild parent. As shown in figure 2, both the upper

[6] In certain crosses with other strains of *A. fatua* some of these correlations have been broken. The nature of these have not yet been sufficiently studied to warrant further discussion.

and lower grain in a spikelet of the wild oats possesses heavy awns. These awns each possess a distinct dark-colored basal portion which extends slightly beyond the tip of the glumes. This basal portion shows a distinct twisting in a clockwise direction. This basal portion is continued into a lighter-colored tapering distal portion which is bent at nearly right angles to the basal portion. This distal portion is approximately twice the length of the basal section. The above description applies to the awns on both the upper and lower grains, but the awn on the upper grain is absolutely somewhat smaller than that on the lower. These awns are invariably present on both grains of every spikelet.

As already noted the F_1 plants present an intermediate condition between the two parents. Awns are present on the lower grain of many spikelets but *never* on the upper grain. The awns on the lower grain vary widely on different spikelets and different plants but they are never so heavy as on the wild. In some cases there is evidence of a basal portion but without any twisting. The distal portion may stand at a slight angle with the basal but does not approach a right-angle. The majority of F_1 spikelets show no awn whatever.

In the F_2 and F_3 generations all plants with the *wild base, show strong twisted and geniculate awns on both grains of every spikelet* quite similar to those of the wild parent. On plants with cultivated or intermediate bases the *upper grain never bears an awn.* On the lower grain the awns vary all the way from none on any of the spikelets to a condition where the awns are almost if not quite as strong as on the lower grain of the wild. However, these awns do not occur on all the spikelets of a plant. On some plants there will be only one or two grains showing strong awns. Other plants may show 90 percent of the spikelets with awns. However, no plant in this cross has yet been found with a cultivated or intermediate base and with all of the spikelets awned.

In describing the condition of the awns on the lower grain of the hybrid plants, the following classification was used. The numbers correspond to the code as punched on the sorting machine cards. (0) absent; (1) weak awns on few spikelets only; (2) same but on 50 percent or more of the spikelets; (3) medium strong awn but without distinct basal and distal portions, on few spikelets only; (4) same but on 50 percent or more of the spikelets; (5) strong awn with distinct distal portion but not geniculate, on few spikelets only; (6) same on 50 percent or more of the spikelets; (7) very strong, geniculate and twisted awns, the distal portion bent at an angle of from 30° to 45° with the basal portion, on few grains only; (8) same but on 50 percent or more

of the spikelets; (9) very heavy, geniculate and twisted awns on all spikelets (wild condition).

In taking this data it was impracticable to examine every spikelet on every head of a large plant. Instead a single head from each plant was examined and the plant recorded accordingly. In this way no doubt some errors have been made. For example, it is extremely easy to overlook one or two weak awns on a large head. Further the head selected might show no awns, while another head on the same plant might have shown one or two weak awns. There is thus a possibility that the class, no awns (0), is larger than it should be.

With this in mind we may turn to the data. Four hundred and sixty F_2 plants have records regarding the awns on the lower grains. The distribution of these according to the above code is given in table 6.

TABLE 6

Awn class	0	1	2	3	4	5	6	7	8	9
Observed No.	133	70	0	74	4	43	6	9	9	112
Grouped No.	133	70		78		49		18		112

In the lower line of the table the classes 1 and 2, 3 and 4, etc., have been grouped together in order to represent the distribution of the kinds of awns without regard to their frequency on the plant. The 112 plants in class 9 are the wilds. The class of greatest frequency is that with no awns. Of the intermediate condition classes 3 and 4 show the greatest frequency. These have a medium strong awn without a distinct basal portion.

The figures in the above table can be grouped in several different ways. Thus the 112 plants in class 9 compared with the 348 plants in the other classes represent a 1 to 3 ratio. This distribution shows complete linkage with the wild and cultivated character of the base (Cf. table 4).

For various reasons that will appear later it seems that a logical grouping is the presence and absence (class 0) of awns. There are then 133 plants without awns to 327 with awns. The expectation on a 1 to 3 basis is 115 to 345. There are 18 more plants recorded without awns than expected. While this number is not beyond the possible fluctuations of sampling, it is probably due in part to errors in classification as discussed above. There seems to be at least some evidence that the presence and absence of awns is due to a Mendelian gene.

The evidence so far then supports the view that awning is controlled

by one principal pair of genes. Plants homozygous for one of these genes possess no awns whatever, while those homozygous for the second member of the pair possess the wild type of awning. The heterozygotes possess awns in various intermediate degrees. These latter differences are probably controlled by secondary minor factors, but the data are not extensive enough to warrant a discussion of these. The observed and expected numbers on the above hypothesis are as follows:

	No awns	Intermediate awns	Wild awns
Observed	133	215	112
Expected	*115*	*230*	*115*

The agreement is fairly good. However, in connection with this it is worth while to examine the F_3 generation. On this assumption the plants without awns ought to breed true to this condition. Of the 20 rows (each with 10 or more plants) grown from mother plants without awns, five bred absolutely true to this condition, while 15 showed some splitting. Three of these 15 rows gave 18 wild plants out of a total of 47. The remaining 12 rows gave 175 plants without awns to 47 plants with intermediate awns. In all of these cases the grain from the mother plant has been re-examined and the classification as to awning appears to be correct.

Thirty-three rows were grown from plants with intermediate awns (classes (1) to (8)). With one exception every one of these rows shows some wild plants and also some plants with no awns.

The fact that the F_2 awnless plants do not all breed true to this condition indicates that possibly other genes are concerned in the production of awns. However, another explanation is available. NILSSON-EHLE (1914) has shown that external conditions are able to greatly affect the production of awns in cultivated oats. General observations also indicate that this character is very variable even within a pure line. It may then happen that plants which are genetically awned plants will, because of environmental or other conditions, show no awns. If seed from such an awnless F_2 plant is planted, the progeny will very probably be awned. Thus in the F_3 data there are seven rows from mother plants classed as number 1 with respect to awns. That is, these mother plants have very weak awns on only a few spikelets. Yet all but one of these seven rows gave some wild plants showing that it was heterozygous with respect to awns and base.

Some further evidence on this point is given by correlating the three classes of awns with the character of the base of the grain. This relation is shown in table 7.

TABLE 7

Distribution of the F_2 plants with respect to awning and the character of the base.

Base	Awns		
	None	Intermediate	Wild
Cultivated	103	13	...
Intermediate	30	202	...
Wild	...	...	112

From this table it is seen that there is an undoubted case of linkage between the character of the base and the character of the awning. If the intermediate condition of the two characters is disregarded the linkage is complete. While there is not sufficient data to settle the question it is quite possible that the absence of awns on plants with an intermediate base is due to the environmental effect on the awns and that genotypically these plants belong to the class with intermediate awns.

It may seem somewhat paradoxical to turn this reasoning about and argue that 13 plants with cultivated bases and intermediate awns really belong to the class with no awns. There are, however, several reasons for believing that this may be true. Each of these 13 plants shows very weak awns on a very few spikelets. In several cases only one awn could be found on the entire plant. Now the cultivated parent in this cross (Kherson, line 26) shows practically no awns. However, in a row of 50 plants, six or eight can usually be found which will show a weak awn on one or two spikelets. Yet we have reason to believe that this line is homozygous for the absence of awns.

Before leaving the character of awning it is of interest to inquire regarding the possible linkage of this character with the color of the grain. NILSSON-EHLE (1914) has shown that in certain crosses and in certain varieties there is a negative correlation between awning and yellow color of the glumes. Table 8 shows the distribution of these characters.

From this table it is seen that while there is a slightly greater proportion of the yellow plants which are awnless than the other colors, yet the difference cannot be regarded as significant. The expected number of awnless yellow plants is 7.25 and the observed number 11. There does not seem to be any marked correlation between these characters in this

TABLE 8

Distribution of the F_2 plants with respect to awning and the color of the grain.

Color	Awns		
	None	Intermediate	Wild
Black	95	161	88
Gray	29	43	15
Yellow	11	9	9

cross. Additional data must be obtained before a final decision can be made.

b. Pubescence on the pedicel

Another character showing complete linkage with the wild base is the presence of pubescence on the pedicel. As shown in figure 3, the wild parent has a very strong pubescence on the pedicel which bears the upper grain. The same is true of the pedicel on the upper grain and even on the third grain if that is present.

Pubescence is entirely absent from the pedicel on the cultivated parent (figure 7) and also on the F_1 grain (figures 9 and 10). It was stated that very infrequently on the cultivated and on the F_1 plants a very few short hairs might be found on the pedicel of the lower grain. None has been observed on the upper grain. This occasional slight pubescence occurs so infrequently and irregularly that no significance has been attached to it.

In the F_2 and F_3 generations the segregation of this character shows complete parallelism with the wild and cultivated base of the grain. Every wild plant regardless of color has pubescence on the pedicel of both the upper and lower grain. Every plant with a cultivated or intermediate base lacks this pubescence. Occasionally an individual F_2 cultivated grain may show a few short hairs on the pedicel just as in the cultivated parent but this appears to occur no more frequently on the hybrids than on the pure cultivated parent.

In the F_2 and F_3 generations of this cross there is not a single exception to this correlation. However, in other crosses, some of which have involved this same line of *A. fatua* and in other crosses with different strains of this species, I have obtained cultivated grains with pubescence on the pedicel. Certain strains of cultivated oats having this pubescence are also known (Cf. NILSSON-EHLE 1911 b). It, therefore, is probable that there is no necessary association of this character with the wild

base and that the results are due to linkage or to the interdependence of factors.

TSCHERMAK (1910) states, apparently on the authority of HAUSKNECHT, that in the second generation of a cross of *A. sativa* × *A. fatua* forms with as little pubescence as that found in cultivated oats are lacking. This is certainly not the case in this cross. Many plants with absolutely no pubescence on any of the grains have been found.

c. Pubescence at the base of the lower grain

On the grain of the wild parent the callus is surrounded on the dorsal and lateral sides with a ring of short stiff hair. On the grain of the cultivated parent this pubescence is entirely absent or occasionally one or two weak hairs are found at the sides of the base. The F_1 plants show an intermediate condition in that there is a tuft of medium long hair at each side of the base but no pubescence on the dorsal side of the base (figure 10 A) on the lower grain. There is no pubescence on the base of the upper grain of any plants except those with a typical wild base.

The F_2 plants show a marked correlation between the character of the base and the kind of pubescence. Thus the 112 plants with wild base all have heavy pubescence extending over the lateral and dorsal sides of the base very similar to the wild parent (figures 12 and 16). The 236 plants with intermediate base have, with one exception, a rather heavy pubescence at the sides of the base but none on the dorsal side. This condition is very similar to that found in the F_1 plants. The one exception noted above has only a very slight pubescence at the sides of the base.

Of the 117 plants with a typical cultivated base, 62 show no pubescence whatever on the base. Fifty-four plants show a slight pubescence at the sides of the base. This pubescence consists of a tuft of only a few hairs at each side. One plant with a cultivated base shows a rather heavy pubescence at the side of the base. The slight pubescence which occurs at the sides of the base is really very little more than is found on some grains of the pure cultivated parent. If we group together the plants with slight pubescence and those with none there is almost complete parallelism between the character of the base and pubescence, as shown in table 9.

The two outlying individuals in this table probably represent random fluctuations of the characters which otherwise show such complete correlation.

TABLE 9

Relation between the character of the base and the pubescence at the base of the lower grain.

Base	Pubescence at base		
	None or slight	Medium heavy but at sides only	Medium heavy on all sides of base
Cultivated	116	1	...
Intermediate	1	235	...
Wild	...	...	112

In the preceding pages a number of apparently distinct characters have been found to be absolutely correlated with the character of the base of the grain. The following table brings together the characters which are always associated with the three types of base.

TABLE 10

Characters correlated with the three types of base on the lower grain.

Cultivated base	Intermediate base	Wild base
Cultivated base on the upper grain.	Cultivated base on the upper grain.	Wild base on the upper grain.
Absence or almost complete absence of awns on the lower grain.	Medium awns on the lower grain of some spikelets.	Very heavy awns on the lower grain of every spikelet.
Total absence of awns on the upper grain.	Total absence of awns on the upper grain.	Very heavy awn on the upper grain of every spikelet.
Absence of pubescence on the pedicel on the lower grain.	Absence of pubescence on the pedicel on the lower grain.	Heavy pubescence on the pedicel on the lower grain.
Absence of pubescence on the pedicel on the upper grain.	Absence of pubescence on the pedicel on the upper grain.	Heavy pubescence on the pedicel on the upper grain.
None or sometimes very slight pubescence at the sides of the base of the lower grain.	Medium heavy tuft of hair at the side of the base of the lower grain.	Heavy pubescence on all sides of the base of the lower grain.
No pubescence on the base of the upper grain.	No pubescence on the base of the upper grain.	Heavy pubescence on all sides of the base of the lower grain.

Here are eight characters which are always associated together. In the wild grain they form an association to which I have so far discovered no exception in this cross. Some of these characters exhibit the same appearance with both the cultivated and intermediate base. Other characters show a difference associated with these two kinds of base. This difference is undoubtedly to be attributed to complete dominance in the first case and to incomplete dominance in the second place. For this reason it appears possible that these several characters are each due to a separate pair of genes rather than to the action of a single pair.

NILSSON-EHLE (1911 a) in his description of some very interesting cases of plants with wild characters, appearing in pure lines of cultivated oats, notes that the characters discussed above always go together. These mutations appeared in a number of different varieties and under conditions in which the possibility of mixing and of natural crossing was out of the question. NILSSON-EHLE regards this group of characters as due to the absence of a single inhibiting factor. It is not desired to discuss this question here but evidence from other crosses not yet completely analyzed, leads the writer to believe that some of these characters at least are controlled by separate genes. In the present data, however, they behave exactly as if due to a single factor.

If the former possibility should prove to be true, the genes must, on the assumption of Morgan and his associates, be located in the same chromosome. These genes must either be located very near each other, or else the oat chromosome does not show the same tendency to "crossing over" that is found in some other organisms. Additional evidence on this point will be brought out in the next section where cases of partial linkage will be discussed.

Characters showing partial linkage

Two characters showing apparent partial linkage to other genes have been discovered in this cross. These characters are (1) pubescence on the back of the lower grain, and (2) pubescence on the back of the upper grain. These two characters are inherited independently of each other, but nevertheless are definitely related to each other as will be shown below.

a. Pubescence on the back of the lower grain

As stated in the early part of this paper, the wild parent is heavily pubescent on the back of each grain (figure 2). This pubescence is longer and heavier in the case of the lower grain. The cultivated parent

lacks this pubescence entirely (figure 4). The F_1 plants are pubescent on the back of the lower grain, but smooth on the back of the upper (figure 8). The pubescence on the lower grain of the F_1 plants is not so heavy as in the wild.

In the F_2 generation the plants which show pubescence on the back of the lower grain segregate from those lacking this character in a 3 to 1 ratio, 347 pubescent to 118 smooth. As shown in table 11 there is no relation between this character and the nature of the base of the grain.

TABLE 11

Relation of pubescence on the back of the lower grain to the character of the base.

	Cultivated		Wild	
	Pubescent	Smooth	Pubescent	Smooth
Observed	257	96	88	25
Expected	*261.5*	*87.2*	*87.2*	*29.1*

In this distribution the cultivated plants without pubescence are somewhat in excess of expectation, but the result is probably due to random fluctuations.

It will be noted from this table that there are two classes of plants here which are different from either parent. (1) A grain with cultivated (or intermediate) base and pubescence on the back of the lower grain (figure 11). In these respects this grain is similar to the F_1 (figure 8). (2) Grain with wild base but without pubescence on the back of the lower grain (figures 12 and 13).

If we attempt to correlate this character with the color of the grain it is seen at once that the results are not in accord with a simple expectation.

Table 12 shows the relation between the genes for black and non-black color and the presence or absence of pubescence on the back of the lower grain. It has already been pointed out that the three color genes, black, gray and yellow, are independent of each other. So far as the present evidence goes this pubescence is related to the black gene and its allelomorph only.

TABLE 12

Relation of the pubescence on the back of the lower grain to the color of the grain.

	Black		Non-black	
	Pubescent	Smooth	Pubescent	Smooth
Observed No.	345	2	0	118

From this table it is seen that there is an almost absolute correlation between the presence of pubescence on the lower grain and the black color. However, two black plants were found which lack entirely this pubescence (figure 14). The grain from these two plants has been carefully examined and no evidence of pubescence could be found. No plants with pubescent non-black grains have been found in this cross. However, in certain other crosses in which this same wild was used, a few non-black pubescent plants have been found. Figure 15 shows a spikelet from such a plant from the cross of wild × Senator. The Senator is a large-grained white variety.

If the results shown in table 10 are due to partial coupling it is clear that the expected number of pubescent non-black plants will be small, not over two or three, out of the 465. Since such plants have been found in other crosses, it seems probable that their absence here is due to a chance fluctuation. Since there is no question as to the correct classification of the two black non-pubescent plants it is very probable that we are dealing here with a case of partial linkage.

It has very frequently been pointed out by various writers (e.g., BRIDGES (1914), STURTEVANT (1915)) that an F_2 generation is not well adapted to the study of linkage. This arises from the fact that in a straight F_2 generation only certain of the crossover zygotes will show the crossover character. In a coupling series these are the ones which are homozygous for one or the other of the recessive genes. Where an F_1 double heterozygote is back-crossed with the double recessive every crossover gamete of the F_1 plant will be evident in the zygote. In this latter case the zygotic ratio corresponds exactly with the gametic ratio. However, the difficulties of making back-crosses in sufficient numbers is very great in a plant like oats. Every crossed grain must be manipulated by hand and even under the best of conditions only a comparatively small percent of manipulated flowers ever set seed. By means of some improved methods it is proposed to make back-crosses involving some of these linked characters.

However, it is not impossible to gain some knowledge regarding linkage from an F_2 population. In the present case the numbers are too small to permit more than a very general suggestion.

We may let

B = the gene for black
b = the gene for non-black
P = the gene for pubescence on the back of the lower grain
p = the gene for smoothness on the back of the lower grain.

The F_1 individuals are therefore *BbPp*, forming the gametes *BP*, *Bp*, *bP* and *bp*. The ratio of the F_2 zygotes (table 12) shows that the gametes *BP* and *bp* are formed almost to the exclusion of the other two. In the table there are only two individuals which show the effect of the crossover gametes. It is probable, however, that some individuals showing the effect of the *bP* crossover gametes might reasonably be expected. We might, therefore, very reasonably assume that the number of individuals showing the effect of the crossover gametes *Bp* and *bP* should be three out of 465. If this is the case the F_2 results represent a gametic ratio (in BATESON's sense) of approximately 150 to 1. That is, the gametes, instead of occurring in equal numbers, are formed in the ratio 150 *BP* : 1 *Bp* : 1 *bP* : 150 *bp*.

While the method of obtaining such a gametic ratio from the proportion of F_2 zygotes is perfectly straightforward, it may be of use to indicate how this may be done, for the benefit of those readers who are not working with linkage in an F_2 generation. The method used in the present work has been to compare empirically the observed F_2 numbers with the expected number when different gametic ratios are used. In calculating the expected number of F_2 zygotes from a given ratio of gametes, the following considerations are of value.

In the above example the F_1 individual is *BbPp* which forms the gametes x *BP* : 1 *Bp* : 1 *bP* : x *bp*, so that x to 1 is the unknown gametic ratio. A little calculation will show that the random mating of such F_1 gametes will give in the F_2 generation.

$3x^2 + 4x + 2$	individuals with phenotypic characters	*BP*
$2x + 1$	individuals with phenotypic characters	*Bp*
$2x + 1$	individuals with phenotypic characters	*bP*
x^2	individuals with phenotypic characters	*bp*

From such formula it is very simple to calculate a table showing the ratio of the four phenotypic F_2 classes for different gametic ratios. For example, if $x = 3$, the ratio of the F_2 classes is 41 *BP* : 7 *Bp* : 7 *bP* : 9 *bp*. If $x = 10$, we have 342 *BP* : 21 *Bp* : 21 *bP* : 100 *bp*, and if $x = 150$ we have 68102 *BP* : 301 *Bp* : 301 *bP* : 22500 *bp*.

A table involving the ratios most likely to be met can easily be calculated.[7] In such a table there can also be inserted a column giving the

[7] It is to be understood that the table discussed here applies only to linkage in the form of coupling. Where the gametes enter in the heterozygous condition we get the gametic series in the repulsion form, viz., 1 *BP* : x *Bp* : x *bP* : 1 *bp*. For these cases a separate table can easily be calculated. Compare also the tables given by JOHANNSEN (1913, p. 573).

percent of the F_2 zygotes showing the crossover character to the total number of zygotes necessary to complete the series. For example, in the above paragraph when $x = 3$, there is a total of sixty-four F_2 zygotes necessary to complete the series without fractions. Of these, 14 show the effect of the crossover gametes, i.e., either *Bp* or *bP*. The percent which 14 is of 65, viz., 21.88, may be called the percent of F_2 crossover zygotes. As x increases, this percentage decreases. Thus for $x = 10$, the percentage is 8.68. For $x = 150$, the percentage is 0.66.[8]

With such a table it is only necessary to calculate the percent of F_2 crossover zygotes from the observed data and then compare it with this column of the table. Thus to return to our data, table 12 shows that there are only two crossover zygotes out of 465 individuals. If we accept the figures as they stand the percent of crossover zygotes is 0.43. Comparing this with a suitable table shows that the corresponding gametic ratio is about 240 : 1. As stated above, however, it is probable that some individuals showing the effect of the *bP* crossover gametes may reasonably be expected. If we assume that the number of individuals showing the effect of the crossover gametes should be three out of 465, then the percent of F_2 crossover zygotes becomes 0.65. Comparing this with the ratios in the above mentioned table shows that it corresponds to a gametic ratio of approximately 150 to 1. That is, the F_2 observed ratios may be explained by assuming that the F_1 plants form gametes in the ratio, 150 *BP* : 1*Bp* : 1 *bP* : 150 *bp*. Thus out of every 302 gametes formed, two will be crossovers, or in MORGAN's terminology the percent of crossovers is approximately 0.66.[9]

Table 13 shows the observed and the expected number in each class on the assumption that linkage occurs in the above ratio.

[8] BRIDGES (1914) has used the coefficient of association as defined by YULE (1911) to determine the gametic ratio from F_2 zygotes. This is undoubtedly of value where the gametic ratio is less than 50 to 1, or where very large numbers of individuals are concerned.

[9] It is of interest to note that in a coupling series, where the gametic ratio is 50 to 1, or greater, the value of the "percentage of F_2 crossover zygotes" as defined above is practically the same as the "percent of crossover gametes" used by MORGAN. As a matter of fact, when the ratio is 25 to 1, the difference between the two percentages is only 0.08. For smaller ratios the differences increase rather rapidly. Thus for 10 to 1 the difference is 0.41 percent and for 3 to 1 it is 3.12 percent. Thus for cases where the gametic ratio is large, the percent of crossover gametes can be calculated with sufficient accuracy directly from the F_2 zygotes quite as easily as in the case of back-cross data.

TABLE 13

Showing the relation between the black color and the pubescence on the back of the lower grain.

	Black		Non-black	
	Pubescent	Smooth	Pubescent	Smooth
Observed	345	2	0	118
Expected	*347.3*	*1.5*	*1.5*	*114.7*

Such an hypothesis gives at least a very reasonable agreement between the observed and expected numbers. These results will be discussed further after the next section of this paper.

b. Pubescence on the back of the upper grain

As shown in figures 2 and 6, the back of the *upper* grain of the wild parent is covered with a strong pubescence. Usually the hairs on the upper grain are not so long or so thick as those on the lower grain. The cultivated parent lacks this pubescence entirely. Likewise, as shown in figure 8, the F_1 plants lack this pubescence on the upper grain. It is apparent therefore that this pubescence on the two grains is controlled by separate genes.[10]

In the F_2 generation there are 378 plants without pubescence on the back of the upper grain to 87 which are pubescent. The ratio here is not 3 to 1 but 4 to 1. This indicates that the gene for pubescence on the upper grain is influenced by other genes. It will be remembered that the pubescence on the lower grain is linked with the black color factor (table 10). Table 14 shows the relation of the black color factor to the pubescence on the back of the upper grain.

TABLE 14

Relation between the black color factor and the pubescence on the back of the upper grain.

	Black		Non-black	
	Smooth	Pubescent	Smooth	Pubescent
Observed No.	260	87	118	0
Expected No. on 9:3:3:1 ratio	*261.5*	*87.2*	*87.2*	*29.1*

[10] It will also be noted that while for the lower grain the presence of pubescence is dominant, the opposite is the case for the upper grain.

From this table it appears that in the case of the black grain the smooth and pubescent grain occurs in a 3 to 1 ratio, but in the non-black grain the plants which would normally be pubescent for some reason fail to develop this character. The observed figures very closely approximate a 9 : 3 : 4 ratio. This is evidently not a case of linkage with the color genes. There is something in the non-black plants which prevents the development of pubescence on the upper grain. In all probability it is not the color gene itself which affects this pubescence, but more likely the genes controlling the pubescence on the back of the lower grain. Thus from table 12 it is seen that there were no non-black plants in this generation which were pubescent on the back of the lower grain. Now if we assume that in the absence of the factor for pubescence on the lower grain the factor for pubescence on the upper remains inactive, the results are explained very well. In this sense the gene for pubescence on the lower grain acts as a basic pubescence factor in a manner quite similar to the basic color factor (C) in mice, rabbits, sweet peas, and many other organisms.

We can further test this hypothesis by ascertaining the relation between the nature of the pubescence on the two grains. This relation is shown in table 15.

TABLE 15

Relation between the pubescence on the lower and the upper grain.

Lower grain	Pubescent		Smooth	
Upper grain	Smooth	Pubescent	Smooth	Pubescent
	258	87	120	0

From this it is seen that again there is a 9 : 3 : 4 ratio. There are no plants which are smooth on the lower grain and pubescent on the upper.

That this relation is between the genes for pubescence on the two grains and does not involve the color gene is indicated by the fact that in other crosses with this same wild form, non-black plants have been found in which both the lower and upper grains are pubescent as shown in figure 15. Other non-black plants have been found in which the lower grain is pubescent and the upper smooth but so far the reverse condition has not been found in any of the crosses examined.

From the above discussion it is evident that the factors for pubescence

on the back of the upper grain are not linked in the ordinary sense, either with the color factor or the factor for pubescence on the lower grain.

In table 16 is shown the relation between the pubescence on the back of the upper grain and the character of the base of the grain.

TABLE 16

Relation between the pubescence on the back of the upper grain and the character of the base.

	Cultivated		Wild	
	Smooth	Pubescent	Smooth	Pubescent
All plants	352	2	27	85
Black plants only	257	2	3	85

It is seen at once that there is distinct evidence of linkage between these two genes. It has been shown that the ratio in the non-black plants is disturbed by interdependence of two genes. Hence it is necessary in considering the question of linkage to use the data from black plants only as given in the second line of the table.

It is to be noted that all expected classes of plants are present although the crossover plants are relatively few. The grain from each of the five crossover plants has been carefully re-examined and there is no question of the correctness of the classification.

If we let

C = cultivated base
c = wild base
S = smooth upper grain
and s = pubescence on upper grain

the gametes containing CS and cs are formed much more frequently than the other two. Calculating the probable gametic ratio to produce such an F_2 generation (using the black plants only) it is found that the ratio is approximately 65 CS : 1 Cs : 1 cS : 65 cs corresponding to 1.52 percent of crossovers. Table 17 shows the observed and expected number of F_2 plants (blacks only) on the assumption that the above linkage takes place.

TABLE 17

Relation between the pubescence on the back of the upper grain and the character of the base (black plants only).

	Cultivated		Wild	
	Smooth	Pubescent	Smooth	Pubescent
Observed No.	257	2	3	85
Expected No.	*257.6*	*2.6*	*2.6*	*84.2*

The hypotheses proposed explain the observed results very well. It is possible to make other assumptions which will also explain these results, but those which have been tested out are either much more complex than the present one or else involve the assumption of inhibiting factors. It seems that wherever possible it is desirable to avoid the latter assumption.

To sum up the main features of the present hypothesis, it is found that the gene for pubescence on the back of the upper grain segregates independently of the gene for pubescence on the back of the lower grain. However, the former gene is unable to produce pubescence unless the factor for pubescence on the lower grain is present in the same zygote. The gene for pubescence on the back of the lower grain shows partial linkage with the gene for black color. The gametic ratio is apparently about 150 to 1. The gene for pubescence on the back of the upper grain is partially linked with the gene for the wild base. The gametic ratio in this case is approximately 65 to 1. It is very probable that these gametic ratios will be changed somewhat when larger numbers of individuals are available. It is believed that the present assumption represents the essential facts.

Some further evidence as to the adequacy of the present assumption can be obtained by comparing in a single table the theoretical and observed frequency of the several phenotypic classes of individuals to be expected in the F_2 generation when all four pairs of genes are considered.

With four pairs of genes 16 phenotypic classes are to be expected in the F_2 generation. However, since pubescence cannot occur on the upper grain unless the gene for pubescence on the lower grain is present in the zygote, there are only 12 classes which can be expected. Table 18 shows the expected classes. The upper of the two rows of figures opposite the heading "theoretical frequency" shows the relative number of individuals expected in each of the 16 possible classes under the linkage postulated above. The lower of these two rows of figures shows the relative frequency in the 12 classes which may be expected. It will be noted that

some of the expected classes on this assumption will occur only once in 40,221 individuals.[11] The upper of the two remaining rows of figures shows the actually observed number of individuals in each class. The bottom row of the table shows the number of individuals to be expected in each class in a total of 465.

TABLE 18

Showing the nature and the frequency in each phenotypic class of individuals of an F_2 generation when the four pairs of genes which show partial linkage are concerned.

Color	A. Black							
Base	Cultivated				Wild			
Pubescence on lower grain	Pubescent		Smooth		Pubescent		Smooth	
Pubescence on upper grain	Smooth	Pubescent	Smooth	Pubescent	Smooth	Pubescent	Smooth	Pubescent
Theoretical frequency	22313 22313	211 211	99 100	1 —	211 211	7297 7297	1 33	32 —
Observed No.	255	2	2	—	3	85	0	—
Expected No.	*257.96*	*2.44*	*1.16*	—	*2.44*	*84.36*	*0.39*	—

Color	B. Non-black							
Base	Cultivated				Wild			
Pubescence on lower grain	Pubescent		Smooth		Pubescent		Smooth	
Pubescence on upper grain	Smooth	Pubescent	Smooth	Pubescent	Smooth	Pubescent	Smooth	Pubescent
Theoretical frequency	99 99	1 1	7372 7442	70 —	1 1	32 32	70 2481	2411 —
Observed No.	0	0	94	—	0	0	24	—
Expected No.	*1.14*	*0.02*	*86.04*	—	*0.02*	*0.37*	*28.68*	—

[11] This "theoretical frequency" was obtained by forming a square table in the usual manner and entering the gametes with their linkage frequency, remembering that the probability of the occurrence of genes in the same gamete is the product of their independent probability. For example, the relative frequency of the *BPCS* gametes is $150 \times 65 = 9750$. We thus have 9750 *BPCS* : 150 *BPCs* : 150 *BPcS* : 9750 *Bpcs* : 65 *BpCS* : 1 *BpCs*, etc. This gives rather large numbers in certain squares, e.g., sixteen squares of the table will have a relative frequency of 9750^2. The theoretical frequency as given in table 18 was obtained by reducing the ratio to its lowest terms.

From this table it is seen that the agreement between the observed and expected number of individuals is very good in nearly all the classes. The slight discrepancy in the non-black classes is not greater than might occur in random fluctuations.

Of the 12 expected classes only seven have been found. However, the total expected frequency of the five classes not represented is only 1.94 individuals out of 465, or an average of less than 0.4 individuals per class. Consequently it is hardly to be expected that any of these classes should be represented in a generation of this size.

On the whole it appears that from the present data it would not be possible to obtain a simpler or more satisfactory explanation of the observed ratios than that offered by the present theory.

It is clear from the number of classes expected in the F_2 generation that a straight F_3 generation containing a relatively small number of rows would be of little aid in determining the correctness of these hypotheses. So far as the F_3 results go they bear out, with one possible exception, the deductions from the F_2 generation.

Out of 28 rows splitting into blacks and non-blacks three give one black plant each, without pubescence on the back of the lower grain. However, there are no non-black plants with pubescence on the lower grain. Thus out of the 445 plants in these 28 rows there are then only three crossovers. However, since the gametes enter the F_2 plants in several different relations it is useless to speculate as to how nearly such a small number of rows agrees with expectation. One row was found with the constitution *BBppCCSS*(or *-Ss*) throwing all black cultivated plants but lacking pubescence on the back of the lower grain.

The absence of non-black individuals with pubescence on the back of the lower grain in the F_3 as well as in the F_2 generation suggests that possibly the interpretation proposed has not taken all of the complications into account. However, a casual inspection of the expected number of plants of this class in a random F_3 generation indicates that this class of crossover plants will occur less frequently than the other. Since only three plants occurred in the latter crossover class, it is hardly to be expected that representatives of the former class would be found. At least there is no evidence here to cause us to change our present hypothesis. Crucial experiments to test this point are now under way.

Regarding the pubescence on the back of the upper grain there are 35 rows splitting into cultivated and wild plants. For the reasons given above only the black plants are of use in determining this relation. These rows give a total of 325 black plants. It has been shown by BATESON

and PUNNETT (1911), as well as by MORGAN (1911), that the linked genes tend to maintain the same relation to each other as that in which they entered the mother plant. It is clear, therefore, that in some F_2 plants the linkage will be the same as in the F_1 plants, viz., 65 *CS* : 1 *Cs* : 1 *cS* : 65 *cs*. In other F_2 plants the linkage will be the other way and will give a repulsion series, viz., 1 *CS* : 65 *Cs* : 65 *cS* : 1 *cs*. Table 19 gives first the observed number of these 325 plants which fall in each class and second the expected number calculated by taking account of the manner in which the gametes enter the F_2 plants and weighting the results with the relative frequency of the several F_2 classes.

TABLE 19

Relation between the base and the pubescence on the back of the upper grain in the black plants from F_3 rows which split into cultivated and wild.

	Cultivated		Wild	
	Smooth	Pubescent	Smooth	Pubescent
Observed	234	8	5	78
Expected	*237.9*	*4.7*	*3.6*	*77.8*

In general there is a fair agreement but the two crossover classes are relatively too large. The expected number is calculated on the supposition that the different classes of F_2 plants were represented in a random sample. In such a small number of rows (35) this might not be the case and consequently some differences in the ratio may be expected.

In addition it may be noted that one row was found coming from a mother plant of the constitution *BbPpccSS*. This gave black and non-black plants, all smooth on the back of the upper grain and all wild.

While the F_3 generation adds little positive evidence, it does not furnish any data which are incompatible with the theory proposed.

The brilliant work of MORGAN and his collaborators upon linkage and its relation to the chromosome theory of inheritance makes it exceedingly attractive to point out the possible relation of these genes to the chromosomes. In the first place it has been shown by NILSSON-EHLE (1909) and partly by the present work that the three pairs of color genes segregate independently of each other. It may, therefore, be supposed that they lie in separate chromosomes. Further the gene for the character of the base of the grain segregates independently of the color genes. It,

therefore, probably lies in a fourth chromosome. The seven characters given in table 8 (p. 269) are very closely if not absolutely linked with the character of the base. If these seven characters may be supposed to be due to separate pairs of genes these must lie in this fourth chromosome. Further the loci of these genes in the chromosome must be relatively close to each other and to the locus of the gene for the base of the grain.

Still a ninth pair of genes is located in this fourth chromosome, viz., that for smoothness (lack of pubescence) on the back of the upper grain. This gene is apparently located at a slight distance from the group discussed above since it shows about 1.5 percent of crossovers with the members of that group.

The gene for pubescence on the back of the lower grain is linked with the gene for black color and is, therefore, to be regarded as located in the same chromosome. The percent of crossovers between these two genes is probably less than 0.7 percent.

SUMMARY

The wild parent used in this cross possesses the following glume characters which are considered in this paper. The flowering glumes are black or dark brown in color. Both the lower and upper grains of each spikelet bear heavy geniculate and twisted awns. The back of each grain is covered with a thick pubescence. Likewise there is a thick ring of short hair about the base of each grain. The pedicel on each grain is heavily pubescent. The base of each grain is expanded into a broad sucker-like ring which permits the grain to shatter very easily (figures 2, 3 and 4).

The grain of the cultivated parent is yellow in color. It possesses no awns except that occasionally a single spikelet may be found with a very weak awn on the lower grain only. The cultivated parent entirely lacks pubescence on all of the regions noted above. The base of the grain is narrow and contracted and the grain does not shatter (figures 6 and 7).

The F_1 plants are generally intermediate between the two parents. The grain is brown in color but distinctly lighter than the wild parent. Medium heavy awns are present on the lower grains of some spikelets. No awns are ever found on the upper grains. The lower grain is pubescent on the back but the upper grain is entirely smooth. There is a fairly heavy tuft of hair at the sides of the base of the lower grain, but none on the upper grain. The base of the lower grain is intermediate in character but more closely resembles the cultivated parent. It does not shatter. The base of the upper grain is like the cultivated parent.

Data are available from 465 F_2 plants. Of these about 70 have been grown in an F_3 generation.

The data show that the wild parents carry genes for gray and probably for yellow color in addition to the black. These three colors segregate independently of each other. The observed ratio closely approximates the expected and confirm NILSSON-EHLE's conclusions.

The cultivated base of the grain is dominant to the wild and segregates independently of the color genes. The heterozygous condition in the lower grain can be recognized in the majority of plants.

In this cross seven pairs of characters are completely correlated with the character of the base. The characters associated with the wild base are (1) heavy awn on the lower grain, (2) awns on the upper grain, (3) wild base on the upper grain, (4) pubescence on the pedicel on the lower, and (5) on the upper grain, (6) pubescence on all sides of the base of the lower grain and (7) pubescence on the base of the upper grain. There is no evidence in the present cross to indicate whether these characters are due to the action of a single pair of genes or to several pairs completely linked. Evidence from certain other crosses, as yet not completely analyzed, indicates the existence of separate genes for some of these characters.

The gene for pubescence on the back of the lower grain is partially linked with the black color factor. The F_2 generation is too small to determine the exact degree of linkage but indicates that there are about 0.7 percent of crossovers.

The gene for pubescence on the back of the upper grain segregates independently of the color factor except that in the absence of the gene for pubescence on the lower grain the gene for pubescence on the upper is unable to act. In this sense the gene for pubescence on the lower grain is a basic pubescence factor similar to the color factor (C) found in many animals and plants.

However, this gene for pubescence on the back of the upper grain is linked with the wild base. The percent of crossovers indicated by the present data is about 1.5.

The observed and expected results in the F_2 generation when both sets of partially linked genes are considered are given in table 18.

LITERATURE CITED

BATESON, W. and PUNNETT, R. C., 1911 On the inter-relations of genetic factors. Proc. Roy. Soc. B **84**: 3-8.

BRIDGES, C. B., 1914 The chromosome hypothesis of linkage applied to cases in sweet peas and Primula. Amer. Nat. **48**: 524-534.

JOHANNSEN, W., 1913 Elemente der exakten Erblichkeitslehre. pp. xi + 723. Jena: Gustav Fischer.

MORGAN, T. H., 1911 An attempt to analyze the constitution of the chromosomes on the basis of sex-limited inheritance in Drosophila. Jour. Exp. Zool., **11**: 365-413.

NILSSON-EHLE, H., 1909 Kreuzungsuntersuchungen an Hafer und Weizen. pp. 122. Lund.

1911 a Ueber Fälle spontanen Wegfallen eines Hemmungsfaktors beim Hafer. Zeitschr. ind. Abst. u. Vererb. **5**: 1-37.

1911 b Spontanes Wegfallen eines Farbenfaktors beim Hafer. Verh. d. naturf. Vereines in Brünn, **49**: 139-156.

1914 Ueber einen als Hemmungsfaktor der Begrannung auftretenden Farbenfaktor beim Hafer. Zeitschr. ind. Abst. u. Vererb. **12**: 36-55.

STURTEVANT, A. H., 1915 The behavior of the chromosomes as studied through linkage. Zeitschr. ind. Abst. u. Vererb. **13**: 234-287.

VON TSCHERMAK, E., 1910 Fruwirth's Die Züchtung der landwirtschaftlichen Kulturpflanzen. 2. Aufl. **4**: 360-365. Berlin: Paul Parey.

YULE, G. U., 1911 An introduction to the theory of statistics. pp. xiii + 376. London: Charles Griffin & Co.

TRICOLOR INHERITANCE. I. THE TRICOLOR SERIES IN GUINEA-PIGS[1]

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INTRODUCTION

In 1897 FRANCIS GALTON published a paper on color inheritance in the Basset hound. This breed of dog has two color varieties, the "tricolor," an animal with black, tan (also called lemon) and white patches, and the "lemon-and-white," with tan (or lemon) and white patches. Neither of these varieties seemed to breed true. Lemon-and-whites might come from tricolored parents, and they might also have tricolor offspring. The pedigrees of 817 dogs furnished the material upon which the paper was based. GALTON applied his "law of ancestral inheritance," proposed some years before (GALTON (1889), to his data, and found that there was a close resemblance between the expected numbers and those actually

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obtained. GALTON's law of ancestral inheritance as stated by himself (GALTON 1897, p. 402) is as follows:

"The two parents contribute between them on the average one-half, or (0.5) of the total heritage of the offspring; the four grandparents one-quarter, or $(0.5)^2$; the eight great-grandparents, one-eighth, or $(0.5)^3$, and so on. Thus the sum of the ancestral contributions is expressed by the series $[(0.5) + (0.5)^2 + (0.5)^3$, etc.], which, being equal to 1, accounts for the whole heritage."

These results have long been a stumbling-block to those who have tried to put a Mendelian interpretation on all forms of color inheritance. Several attempts have been made to attack the problem by working with guinea-pigs. In 1912 CASTLE published the results of his experiments on tricolor inheritance in these animals. He says (CASTLE 1912, p. 438):

"The tricolor animal is white marked with irregular but distinct blotches of black and yellow. Tricolors produce, besides tricolors, young which are black-and-white or yellow-and-white, but never in my experience those which are wholly free from white. In other words they breed true to spotting with white, but not to spotting with black and yellow. The black-and-white as well as the yellow-and-white offspring of tricolor parents may produce tricolor young. Indeed any of these three conditions is able to produce both the others. Notwithstanding the fact that *neither the black-and-whites nor the yellow-and-whites produced by tricolors breed true,*[2] there are races of black-and-white and of yellow-and-white guinea-pigs which do breed true. It remains to explain why the others do not. A black-and-white animal which breeds true may be considered to possess some chemical substance necessary for the production of color (which we call a color factor) distributed irregularly throughout its coat. Wherever this substance is wanting no color is formed and a white area results. The specific factor for black (probably an enzyme) is however everywhere present in the coat, so that wherever color forms, the color is black. Such races as this breed true.

"The yellow-and-white animal which breeds true may likewise be considered to have an irregularly distributed color factor, but to lack entirely in its coat the black factor. Hence the color, wherever formed, is yellow.

"Yellow races also exist which do not bear spots of white, but which have spots of black. In such animals [commonly known as tortoises] the color factor is evidently uniform in distribution, whereas the black factor is irregularly distributed.

"Now the tricolor race is a yellow one spotted both with white and with black, i.e., it results from irregularity in distribution through the coat of two different chemical substances, the color factor and the black factor. These two factors are known to be independent of each other in heredity. See CASTLE (1909). It is therefore not to be supposed that they will coincide in distribution. If the black factor extends over all the colored areas, the animal will be black-and-white. If the black factor falls only on areas which lack the color factor, it will produce no visible effect, and the animal will be yellow-and-white. If, finally, the black factor falls on some

[2] Original not italicized.

of the colored areas, but not on all of them, those in which it falls will be black, the others yellow, and the uncolored areas of course white. Hence a tricolor will result. But the gametic composition of these tricolors will not be different from that of the black-and-whites, or red-and-whites, produced by the same race, since all alike will be characterized by irregularity in distribution of the same two factors. A tricolor race on this hypothesis should be unfixable, as has up to the present time been found to be true."

Although CASTLE was able to pick out the different characters concerned and to show their relationship to each other, his results show that a finer analysis is necessary in order to prove that we are dealing with Mendelian allelomorphs. In his table giving his results he takes for granted that all tricolors are alike and accordingly gives the offspring from such matings with their percentage of tricolors. He treats the red-and-whites and black-and-whites in a similar manner. It is the object of the present paper to demonstrate that there are two kinds of tricolors, three kinds of red-and-whites and two kinds of black-and-whites. By dividing the three color varieties into these different classes it is possible to show relatively good Mendelian ratios in the offspring.

GOODALE and MORGAN (1913) have also published a paper on the inheritance of the tricolor coat in guinea-pigs. They followed methods similar to those employed by CASTLE, but in addition mated some of their tricolors to self ("uniform") reds and self blacks. They made the observation that when the agouti factor is present in a tricolor, the black spots are always affected and as a result we have an animal with white, red and agouti spots.[3] They objected to CASTLE's hypothesis of overlapping areas as an explanation for the formation of the three different color varieties, because it seemed very improbable to them that mere chance would account for the proportionately large numbers of red-and whites, and black-and-whites of tricolor parentage. In their concluding paragraph they state,

"Our matings show that the distributer for black is dominant, as seen in tricolor by uniform, and tortoise [black-and-red] by uniform, giving tortoise, and tricolor by tricolor giving bicolor black [black-and-white]. On this hypothesis our original race of tricolors must have been heterozygous for the black distributer, and hence could throw some bicolor blacks which are real bicolors, not overlapped bicolors. This explains our excess of bicolor black which belonged to both types."

From the foregoing paragraphs it is clear that the great difficulty in an analysis of the guinea-pigs in what may be termed the tricolor series (comprising black-and-whites and red-and-whites of tricolor parentage

[3] The same thing is true of the chocolate spots, which become "cinnamon-agouti." This follows from the fact that the agouti factor produces a visible effect on black and chocolate pigment, but not on red.

as well as tricolors themselves) lay in the masking of the real relation of black to red by the more or less complete overlapping of these areas by white. In other words every animal in the tricolor series is in reality a tortoise in which the black-red pattern is partially obscured by the white markings. Ordinarily the white covers only a part of the black and a part of the red, and the result is a tricolor; if all the red areas are completely overlapped it is a black-and-white; if the black happens to be completely covered a red-and-white results. It should be remembered that in each case identically the same factors are concerned, the difference in appearance depending altogether on the distribution of the red and black pigmented areas and the non-pigmented (white) areas. It is apparent that if the white could be eliminated the study of the black-red relationship would be greatly facilitated. This result can be accomplished indirectly by mating the animals of the tricolor series to homozygous self reds, as a consequence of which all the offspring will be entirely pigmented and the distribution of red and black can be observed directly. Breeding to self reds constitutes therefore the best test of the real composition of any animal spotted with white, and is the method upon which we have based our analysis in the present investigation. As a result we have found, as has been said, that when the facts are fully analyzed the various matings of the different color varieties result in ordinarily good Mendelian ratios.

Before proceeding further, it seems desirable to name and describe accurately the factors involved. At the outset it may be stated that there are no new factors to name or describe, all of them having been designated by CASTLE in previous papers. In some cases it has seemed advisable to modify or to amplify the description of these factors, and in three cases to employ different symbols for them.

FACTORIAL ANALYSIS

Factors always present

When we leave out of consideration some of the newer, and therefore uncommon, color varieties recently described by CASTLE (1914), we may say that there are two factors which are always present in all color varieties of the guinea-pig. These are *R*, the factor for red, and *C*, the factor for chocolate or brown.[4] When unmodified by other factors, red is found

[4] CASTLE uses *C* as the symbol for the color factor and *Br* as the symbol for brown or chocolate. As there is an obvious advantage in having only one letter to represent a factor, it was thought advisable to use *P* (pigmentation) as the symbol for the factor for color and *C* as the symbol for the factor for chocolate or brown.

throughout the skin and coat of the entire animal, while chocolate, under like conditions, is restricted to the eyes, eyelids, and the skin of the ears and feet. Every self red animal, therefore, must carry chocolate. Since both these factors are always present they are commonly assumed, and accordingly left out, in writing the factorial composition of the animals.

Other factors

B is the factor for black. Like *C*, the factor for chocolate, it is restricted to the eyes, eyelids, and to the skin of the ears and feet when unmodified by other factors. Black is epistatic to chocolate.

S represents the factor for *self* pigmentation, meaning that the entire coat is pigmented,—there are no white spots. It is allelomorphic to spotting with white. Tortoises, although not uniformly pigmented, must, nevertheless, carry the factor *S;* they may however be heterozygous for it (*Ss*), in which case, when inbred, they throw some offspring spotted with white (*ss*), as was demonstrated by GOODALE and MORGAN (1913). Although *S* and its absence are treated as simple Mendelian allelomorphs in the present paper, evidence is accumulating which seems to indicate that the relationship is not as simple as has hitherto been supposed. It will not, however, vitiate results to consider *S* at this time as a simple dominant to its absence.

E is the extension factor. When present it extends the black or the chocolate throughout the coat, giving a uniform black or a uniform chocolate. In the absence of *S*, as a result of which the animal is white spotted, pigment formation fails where the white spots occur and we therefore have black or chocolate animals with white spots. Black-and-whites or chocolate-and-whites, homozygous for *E*, will necessarily breed true.

E′ was used by CASTLE (1909) for a factor which extends the black or the chocolate pigment only partially through the coat, thereby forming relatively large black or chocolate patches. He also mentions an *E″* factor which causes the black or the chocolate to be extended in small numerous spots. None of the animals we have worked with seem to carry this latter factor. *E* is dominant over *E′*, as was mentioned by LITTLE (1913, p. 42), and from facts now at hand, and which will be published soon, it is evident that these factors stand in the relation of allelomorphs to each other, *e* being recessive to both. In order to conform more closely with the terminology used by T. H. MORGAN and his associates, in other cases of multiple allelomorphs, it has been deemed advisable to use the symbol e^p in place of E′, the "^{p}" in this combination referring to a lessened and therefore partial degree of "extension", the

resulting character being spotting with chocolate or black on a red background. In the presence of *S* and in the absence of *E*, an animal carrying e^b is therefore a tortoise, i.e., a red animal with black or chocolate patches. In the absence of *S* under the same conditions we have a tricolor. Both Ee^b and *Ee*, in the presence of *S* give uniform self black (when *B* is present) or uniform chocolate (in the absence of *B*). It is not improbable that the type with smaller and more numerous spots referred to above will be found to fall in this same series of multiple allelomorphs, but this has not as yet been determined.

The accompanying table (table 1) shows concisely the various color types produced by the different possible combinations of the factors which have been defined in the foregoing paragraphs, and reference to it will facilitate the discussion which is to follow. It may be of interest to note that all the types mentioned have been produced in our laboratory. Figure 1 represents diagrammatically the color relations of the types carrying *B* (black). These diagrams would do equally well for the chocolate series (*b*), since in the absence of *B* the chocolate would occupy exactly the black areas.

EXPERIMENTAL RESULTS

In the present discussion it may greatly simplify matters when tabulating results to make no distinction between black and chocolate. Animals of the formula e^be^b or e^be are spotted with melanic pigment, either chocolate or black. The spots are of chief interest.

The results obtained from the various matings will be given in the following pages. We shall first attempt to show that there are two kinds of tricolors, (1) those homozygous and (2) those heterozygous for e^b, the black-spotting factor.

Tricolor × self red

Formula No. 7 (see table 1) is that of a tricolor. It is evident that two kinds of tricolor zygotes may be formed, i.e., one that is homozygous for e^b, and another kind that is heterozygous for the same factor, viz., e^be. Both kinds have been obtained. The method employed in testing them has been to mate them with uniform reds. In the former case all the offspring should be tortoise, while in the latter we should expect approximately half tortoises and half self reds, as follows:

Tricolor (type 7), e^be^bssBB × self red (type 10), *eeSSbb* = tortoise (type 5), e^beSsBb;

Tricolor, e^bessBB × self red, *eeSSbb* = 1 tortoise, e^beSsBb : 1 self red, *eeSsBb*.

TABLE 1

Constant factors	Allelomorphic series	Alternative allelomorphs		Type No.	Phenotypic formula	Phenotypic appearance
R (red) C (chocolate)	E (complete extension of melanic pigment)	S (complete pigmentation)	B (black present)	1	(RC)ESB	= self black
			b (black absent)	2	(RC)ESb	= self chocolate
		s (spotting with white)	B (black present)	3	(RC)EsB	= black-and-white
			b (black absent)	4	(RC)Esb	= chocolate-and-white
	e^p (partial extension; spotting with black or chocolate)	S (complete pigmentation)	B (black present)	5	(RC)e^pSB	= tortoise (black-and-red)
			b (black absent)	6	(RC)e^pSb	= tortoise (chocolate-and-red)
		s (spotting with white)	B (black present)	7	(RC)e^psB	= tricolor (white-black-and-red)[a]
			b (black absent)	8	(RC)e^psb	= tricolor (white-chocolate-and-red)[a]
	e (non-extension of melanic pigment)	S (complete pigmentation)	B (black present)	9	(RC)eSB	= black-eyed self red
			b (black absent)	10	(RC)eSb	= brown-eyed self red
		s (spotting with white)	B (black present)	11	(RC)esB	= black-eyed red-and-white
			b (black absent)	12	(RC)esb	= brown-eyed red-and-white

[a] Under certain conditions these types may appear as red-and-white or as black(or chocolate)-and-white. See diagrams, figure 1, types 7 *b* and 7 *c*.

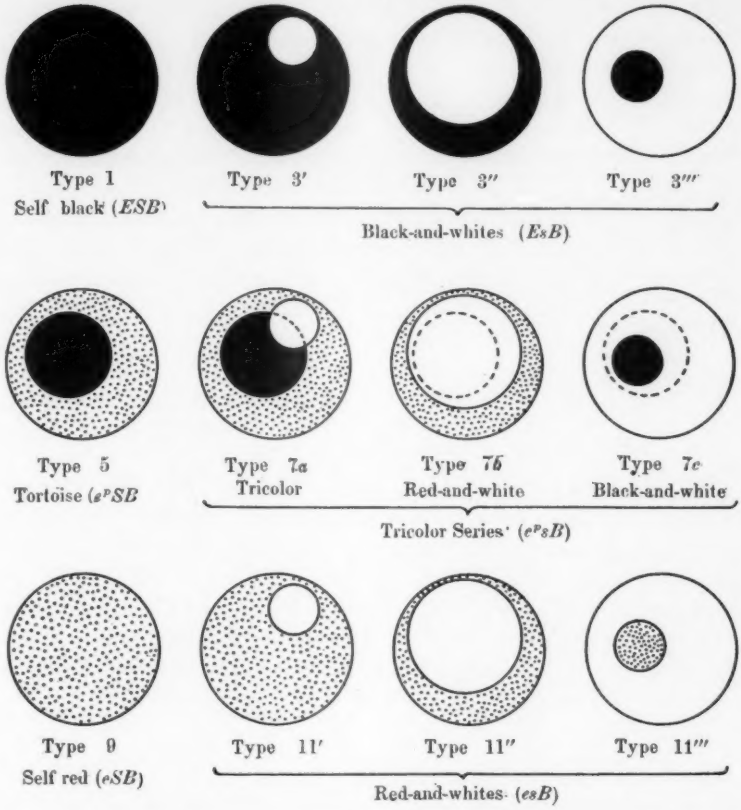

FIGURE 1.—Diagrammatic representation of the color types carrying *B* shown in table 1. Red is represented by dotted areas, black by solid color, and white is left unshaded. In 7 *a*, 7 *b* and 7 *c* the dotted line represents the outline of the black spot shown in type 5. The diagrams in type 7 illustrate how the three appearances, tricolor, red-and-white and black-and-white, may depend entirely upon the distribution of the white. The pairs, 3‴ and 7 *c*, and 11″ and 7 *b* indicate how two animals may be phenotypically alike but genotypically different. Type numbers are the same as in table 1.

Table 2 shows the actual results of the breeding tests. The 81 offspring from the mating homozygous tricolor × self red represent litters from 8 tricolor individuals.

TABLE 2

Tricolors × self reds

Parents	Offspring		
	Tortoise (incl. tricolors)	Self red (incl. *ee* red-and-whites)	Red-and-white (not tested)
Self red × tricolor ($e^p e^p$)	81	0	0
Self red × tricolor ($e^p e$)	37	47	9

This means an average of 10 offspring to each tricolor tested. In no case was the number of offspring for any individual less than 5[6], while in two instances it was 15. Four of the homozygous tricolors were mated to other tricolors but this kind of mating will be discussed later.

Some of the self reds used in the heterozygous tricolor × self red cross were not homozygous for self (*S*) and hence some white spotted offspring resulted. The tricolor and tortoise offspring have been lumped together as "tortoises" because we know they all carry the e^p factor. The red-and-whites, however, may or may not carry the e^p factor and have therefore been classified separately. If those matings in which red-and-whites occur among the offspring were excluded, the numbers would be 33 tortoises and 43 self reds. Whether or not the red-and-whites are eliminated it will be noted that the self reds are in either case considerably in excess. A similar excess of extracted reds has been noted by CASTLE (1905) in guinea-pigs and by COLE (1914) in pigeons.

Tortoise crosses

Some of the tortoise offspring obtained from the tricolor × self red crosses described above were mated together. The zygotic composition of these animals would be $e^p eSs$. We should therefore expect 9 tortoises ($e^p S$) : 3 self reds (eS) : 3 tricolors ($e^p s$) : 1 red-and-white (es) in the offspring. The tricolors differ from the tortoises and the *ee* red-and-whites differ from the self reds only in that they show white spotting (ss). White spotting, however, does not behave as a simple Mendelian recessive. In the first place, when a homozygous self animal is crossed with a white-spotted one we have in no case obtained complete dominance

[6] Five was chosen arbitrarily as a number giving a fair indication of homozygosity. A larger number would have been desirable, but owing to the small litters and the long gestation period in guinea-pigs it is often difficult to obtain more than five offspring from a single individual. Several tricolors, when mated to self reds, had only 1 to 4 offspring, all tortoises, but these seemed too few to establish their homozygosity, and hence the records are not included in the table.

of self in the first generation, since in all such crosses some of the F_1's have shown a small white blaze on the head or one or more white feet. When these slightly spotted F_1's are inbred they produce some offspring like themselves as well as selfs and ordinary white-spotted of the grandparental type. These same classes of offspring are obtained by inbreeding the self-colored animals of the first generation, which makes it appear that the two F_1 types are hereditarily alike.

Another puzzling phenomenon is that sometimes when two extracted white-spotted animals having comparatively large white spots are bred together they get among their offspring some animals that are selfs. From the above considerations it will be seen that there is some difficulty in distinguishing between true self animals and true recessive white-spotted ones. We have therefore in our tortoise crosses disregarded white spotting as much as possible and considered chiefly the presence or absence of e^b, the black spotting factor. When this is done the tortoises and tricolors are put in one class because they carry the e^b factor and self reds and *ee* red-and-whites in the other because they lack it. The expectation then would be 3 tortoises or tricolors to 1 self red or *ee* red-and-white.

Heterozygous tortoises (e^be) similar to those used in the above cross were also mated to self reds. In this case the expectation is an equal number of tortoises and self reds. Here there ought not to be any offspring with large white spots if the self reds used are homozygous for self. Table 3 gives the numbers obtained and also the expected numbers for both kinds of crosses.

TABLE 3

Tortoise crosses

Parent mating		Offspring		
		Tortoise and tricolors	Self reds and *ee* red-and-whites (tested)	Red-and-whites not tested
Tortoise (e^be) × Tortoise (e^be)	Actual numbers	64	29	9
	Expectation	*76.5*	*25.5*	
Tortoise (e^be) × Self red (ee)	Actual numbers	31	46	
	Expectation	*38.5*	*38.5*	

An inspection of Table 3 shows that there are two kinds of red-and-whites listed, those "tested" and those "not tested." As previously stated, red-and-whites may or may not carry the e^p factor. This point can be determined by mating these animals to self reds as will be more fully discussed later. In our e^pe tortoise $\times$ e^pe tortoise cross 12 red-and-whites were obtained. Of these, 9 died before they had any offspring, or at any rate before they had enough to make certain of their composition. The above-mentioned 9 guinea-pigs constitute the red-and-whites "not tested." The remaining 3 of the 12 proved to be *ee* and therefore were classified with the self reds. The probabilities therefore are that the majority of the red-and-whites not tested do not carry the e^p factor, and this would tend to swell the already disproportionately large number of *ee* animals. As in previous crosses, the number of *ee* animals in the two crosses given in table 3 is considerably above expectation. Up to the present there is no good explanation for this.

It may not be out of place to make mention here of the fact that tortoises vary greatly in the extent to which their coats are made up of black (or chocolate) pigment patches. In a majority of cases these patches constitute more than half of the entire coat. There are animals, however, which have very small patches of black (see figure 3, *f*). On the other hand there are a considerable number that are entirely black except for a few small red spots (see figure 3, *b*). In 3 instances there have been animals born of tortoise parentage that to all appearance were self black. Two of these died young, while the third when mated to self reds gave birth only to tortoises and to self reds. This leads one to the natural conclusion that these "self" blacks are not in reality selfs, but only tortoises in which the black happened to be extended so far that all of the red was covered by it. The fewness of their numbers also points to the same conclusion. Following this same line of reasoning it seems probable that tortoises may be obtained in which the black is so little extended that they are to all appearances *self reds*. None of these have been detected so far, although many extracted reds from tortoise crosses have been bred together.[7]

A consideration of the data given in the above paragraph would make it seem that the e^p (or black spotting) factor is a favorable one for

[7] GOODALE and MORGAN mated together self reds from a tricolor $\times$ self red cross and obtained among the offspring one animal showing a small black spot on its back. This would make it seem that at least one of the parents carried the e^p factor although it was not visibly expressed. WHITING (1915) has given an explanation similar to the one mentioned in the text, to account for the occurrence of self black female cats instead of the expected tortoiseshells in the black ♀ $\times$ orange ♂ mating.

testing the effects of selection on a variable character, and experiments along these lines are now in progress.

Red-and-whites

It is obvious that three kinds of tricolor crosses can be made—one in which both the parents are homozygous for the e^b factor, another in which one parent is homozygous and the other heterozygous, and a third in which both parents are heterozygous. In the first two crosses all the offspring would have the e^b factor, though some, in the second case, would be heterozygous for it. In the third cross the expectation is that one-fourth the offspring would lack e^b entirely, i.e., would be *ee*. If any red-and-white offspring resulted from either of the first two crosses they would be sure to carry the e^b factor, while in the case of the third cross there would always be pure red-and-white offspring (*ee*) and there would also be the possibility of some red-and-whites carrying the e^b factor.

The presence or absence of the e^b factor in red-and-whites must therefore be determined by breeding tests. As has previously been explained the black spotting (e^b) when present in red-and-whites is masked because the white spotting happens to fall where the black patches would otherwise have been (see figure 1, type 7 b). To test the red-and-whites, then, it is desirable to mate them to animals that we know are *ee* and at the same time will cause the offspring to be entirely pigmented, so that if the e^b factor is present in the red-and-white it will be sure to be visibly expressed in the offspring. This result can be effected by mating the red-and-whites to homozygous self reds, which because they are *SS*, will get offspring that are entirely pigmented. If the e^b factor is present in the red-and-white being tested, all the offspring should be tortoises when it is homozygous for the factor, and half of them tortoises and the remainder self reds when it is heterozygous. All should be self reds when the factor is absent.

The above method has been used in testing all of our red-and-whites that lived long enough to be tested. We have not attempted, however, to determine whether or not a red-and-white carrying the e^b factor was homozygous for it. What we were primarily interested in was whether or not the factor was present. If five self red and no tortoise offspring were obtained it was taken as a fair indication that the animal being tested was a pure recessive (*ee*).

Summarizing those matings in which both self red and tortoise offspring were obtained, and in which the red-and-white parent was accordingly $e^b e$, gives 43 self reds and 31 tortoises instead of the expected equality. As usual the *ee* animals are in excess.

A consideration of the above paragraphs will make it apparent that three kinds of red-and-whites occur:

1. Those that are homozygous for e^p } (figure 1, type 7)
2. Those that are heterozygous for e^p }
3. Those that lack it entirely (figure 1, type 11).

Black-and-whites

Black-and-whites of tricolor parentage may be variously described. For the sake of simplicity it is perhaps convenient to look upon them as tortoises (figure 1, type 5) which have had white spotting superimposed upon them in such a manner that the white areas cover up all of the red patches and probably always part of the black as well, leaving a white animal with black spots (figure 1, type 7 *c*). The black spotting in the above kind of black-and-whites is the same as that found in tricolors and in e^p red-and-whites (type 7 *b*). This can be demonstrated by matings with self reds. As before stated, e^pe^p tricolors and e^pe^p red-and-whites when mated to self reds have all tortoise offspring. In a similar manner one of our black-and-whites (♀ 18.1) when mated to self reds had 7 tortoise and no self red offspring. Another black-and-white (♂ 11.1) when mated to *ee* red-and-white females (figure 1, type 11) had offspring consisting of 6 tricolors (e^pe) and one red-and-white, which was tested and found to carry black spotting (therefore also e^pe). The results of the above matings indicate that both of the black-and-whites tested were homozygous for e^p. These animals are in the ancestry of all of our black-and-whites[8] except one, and it is probable therefore that some of the latter when tested would also have been found to be homozygous for the black spotting (e^p) factor. There is no good reason, on the other hand, for assuming that some of the black-and-whites would not have been heterozygous for the factor. If we are justified in the assumption that there are e^pe black-and-whites, then we may state that black-and-whites *of tricolor parentage* are of two kinds:

1. Those homozygous for e^p } (figure 1, type 7 *c*).
2. Those heterozygous for e^p }

No black-and-white can be *ee* because this would mean an animal from which the black spotting factor is absent.

A summary of the black-and-white matings is given in table 4. The fact that no *ee* red-and-white offspring were obtained, strongly indicates that at least one of the parents in every mating was homozygous for the black-spotting factor (e^p). This is in line with what has been stated above concerning the black-and-white animals used.

[8] By black-and-white is meant an animal in which no red spotting whatever is visible. Many of GOODALE and MORGAN's "bicolor blacks" had small areas of red in their coats.

TABLE 4

Black-and-white crosses

Parents	Offspring		
	Tricolors	Black-and-whites	Red-and-whites (e^p) (tested)
Black-and-white × Black-and-white	17	8	
Black-and-white × Tricolor	10	2	
Black-and-white × Red-and-white (ee)	11		1

In the first two types of matings the percentage of black-and-whites is high compared with that in tricolor × tricolor matings (see table 5). This will be taken up later in the discussion.

Tricolor × tricolor

We are now in a position to discuss tricolor × tricolor matings. As previously stated, there should be two kinds of tricolors—those that are homozygous for e^p and those that are heterozygous for it. Homozygous animals bred together should produce offspring, all of which carry the e^p factor. These would not necessarily be tricolors because black-and-whites from tricolor parents *do*, and red-and-whites *may*, also carry this factor. On the other hand tricolors heterozygous for the e^p factor should produce offspring one-fourth of which are red-and-whites that do *not* carry this factor; in addition there may be other red-and-whites which do, as is shown in table 5.

TABLE 5

Tricolors × tricolors

Parents	Offspring						Expectation	
	e^p				ee	Not tested	e^p	ee
	Tricolor	Black-and-white	Red-and-white	Total	Red-and-white	Red-and-white		
$e^pe^p \times e^p,^p$ or e^pe	54	2	4	60	0	2	62	0
$e^pe \times e^pe$	28	0	2	30	6	6	31.5	10.5

In the first cross recorded in table 5 at least one of the parents in each mating had been tested with self reds and had been proved to be homozygous for the black spotting factor. Therefore this factor should be present in all of the offspring. The only offspring about which there could be any doubt would be the red-and-whites. There were 6 of these, 2 of which died before they could be tested. The remaining 4 when bred to self reds proved to carry the e^b factor. It will be noted that the percentage of black-and-whites is quite low in this mating, and there are none at all in the heterozygous tricolor cross. A large number would not be expected because tricolors do not tend to carry as much black spotting as do the black-and-whites.

That both parents were e^be in the second cross recorded in table 5 was determined in one of two ways. Either they were both tested by means of matings with self reds or else it was indicated by the fact that they produced some *ee* red-and-white offspring. All the red-and-white offspring from parents tested in either way were mated when possible to self reds to determine whether or not they carried the black spotting factor. Six of the 14 red-and-white offspring could not be tested because of their early deaths; of the remaining 8 there were two that carried the e^b factor and six that did not.

In both crosses reported in table 5 results fairly close to expectation were obtained.

DISCUSSION

In the present discussion we shall look upon the tricolor series in guinea-pigs as red animals irregularly spotted with black and with white. In the case of the tricolors some of the red is visible; in the black-and-whites the red is partially blotted out by the white spotting and the remainder masked by the black spotting; in the red-and-whites all of the black is blotted out by the white spotting while some of the red shows. This agrees with the explanation given by CASTLE (1912). He, however, assumes that *all* red-and-whites of tricolor parentage carry the black spotting factor, whereas we have shown that *ee* red-and-whites are produced as recessives when both the tricolor parents are heterozygous for e^b. Such red-and-whites breed true, contrary to CASTLE's assumption, quoted on p. 288.

Some matings tend to produce a large percentage of black-and-whites, others a fairly large number of e^b red-and-whites, and still others almost entirely tricolors. One possible explanation is that there may be factors which tend to localize and restrict the black spotting and the white spot-

ting to certain more or less definite regions. Two conditions might result, (1) if the black spots tend to fall on what would otherwise have been red areas the number of black-and-whites will be increased; (2) if the black spots tend to fall in the white areas, thus being blotted out, more red-and-whites will result. Our experience, however, substantiates CASTLE's interpretation that the black and the white spotting are entirely independent of each other and are in no sense localized, i.e., they are not any more liable to fall on one part of the body than on another.[9] Each,

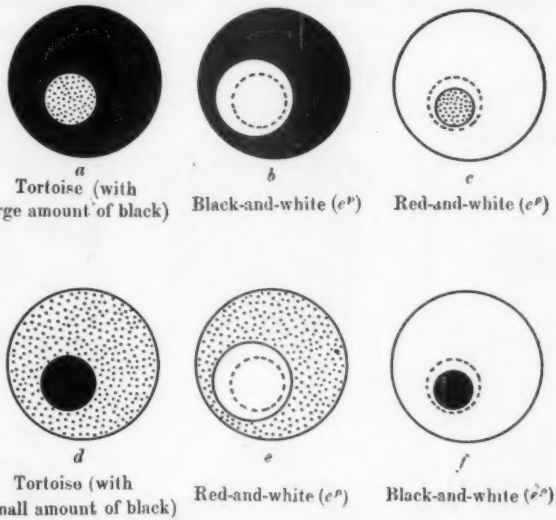

FIGURE 2.—Diagrams showing how the amount of black pigmentation in a white spotted animal may largely affect its chance of becoming a black-and-white or an e^p red-and-white. Only a small amount of white is necessary, rightly placed, to convert *a* into a black-and-white, as shown in *b*; similarly *d* may easily become a red-and-white (*e*). A great deal of white would be necessary, also rightly located (*c*), to make a red-and-white of *a*, and the same would be true to make a black-and-white (*f*) of *d*.

however, may vary in amount. This is shown by the fact that, as previously stated, the amount of black pigmentation in tortoises varies greatly, and the same may be said regarding the amount of white spotting in tricolors. It is due to these variations in the amount of black

[9] Varieties, such as the "Dutch marked," in which white seems to have been fixed in a more or less definite pattern, are not here considered. A white pattern could probably be fixed with some definiteness in tricolors, but this has never been done so far as we know.

pigmentation and the extent of the white areas that some animals which would otherwise be tricolors become black-and-whites and others c^p red-and-whites. The diagrams in figure 2 may be of service in explaining this point. They demonstrate the fact that if an animal carries a large amount of black pigmentation (*a*) the chances are that the red areas will be blotted out by the white spotting (*b*) oftener than will the black areas (*c*). The larger the white areas the better the chances that the red will be blotted out and that black-and-whites will result. In the same way a small amount of black pigmentation (*d*) and a large amount of white spotting facilitate the production of c^p red-and-whites (*e*), and a black-

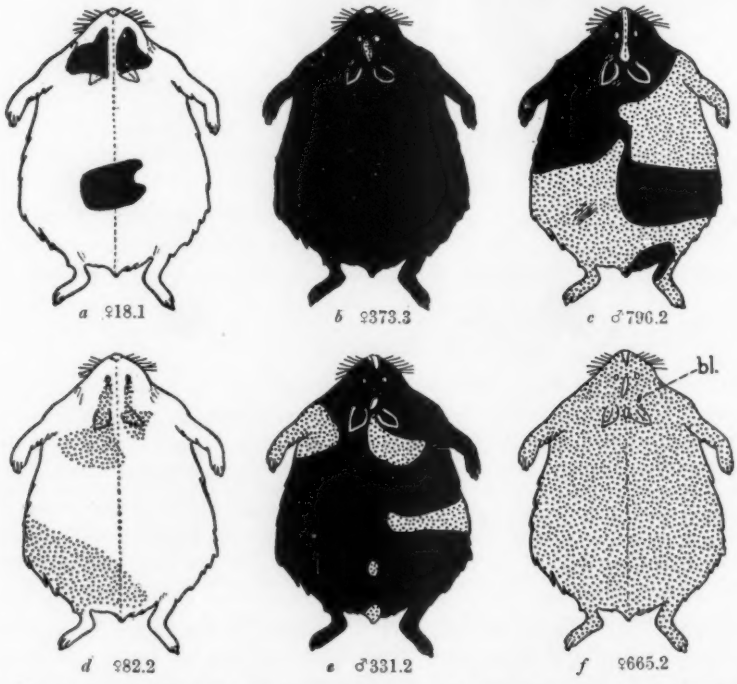

FIGURE 3.—Outline drawings of an c^p black-and-white female, *a*, an c^p red-and-white female, *d*, and two tortoise offspring of each resulting from matings with self red males. The opposite extremes of black pigmentation occurring in the tortoise offspring of *a* are represented by *b* and *c*. In like manner *e* and *f* represent the extremes of black pigmentation in the 8 tortoise offspring of *d*. (Figure 3, *b* is really an agouti-and-red, due to the fact that the self red male carried the agouti factor, but for the sake of simplicity it has been represented in the drawing as a black-and-red.)

and-white may be produced under these conditions when the amount of white is large enough and is properly placed (*f*).

What evidence have we that such is the case? In other words, can it be demonstrated that an e^{ℓ} black-and-white, for instance, usually carries a large amount of black spotting and an e^{ℓ} red-and-white a small amount? This cannot be determined directly by physical examination of the animals in question, but it can be approximately determined by mating the animals to be tested to self reds and thus obtaining tortoise offspring. If the tortoise offspring from a black-and-white × self red cross carry more black on the average than do the tortoise offspring from an e^{ℓ} red-and-white × self red cross, we are justified in assuming that the

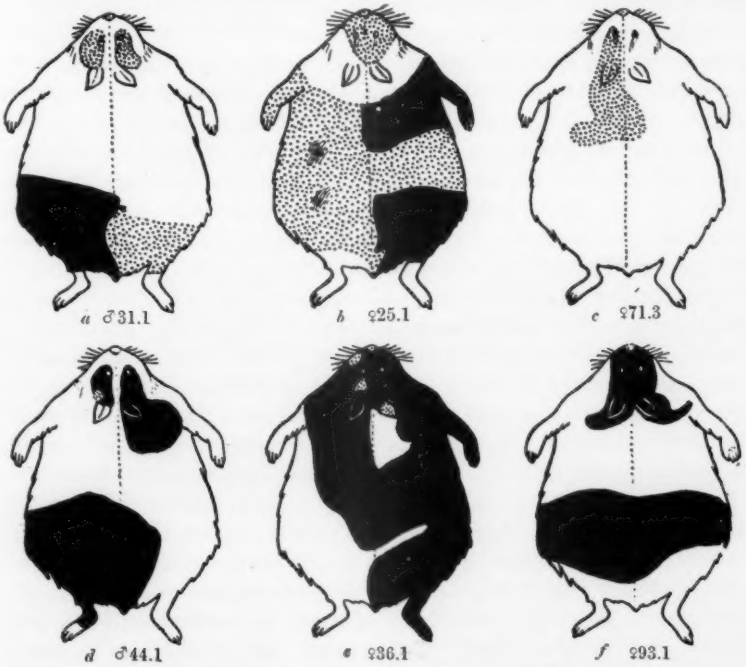

FIGURE 4.—Outline drawings of two pairs of tricolor guinea-pigs (*a* and *b*, and *d* and *e*) and one characteristic bicolor offspring of each (*c* and *f*). The pair *a* and *b*, showing a relatively small amount of black pigmentation, had 5 offspring. Of these 3 were tricolors and the other 2 were e^{ℓ} red-and-whites. Diagram *c* represents one of the latter. The pair *d* and *e*, showing a relatively large amount of black pigmentation, had 8 offspring. Of these 7 were tricolors and one was a black-and-white. Diagram *f* represents the latter.

black-and-white probably carried more black pigmentation than did the e^p red-and-white. Figure 3 gives outline drawings of animals actually tested in this manner, and certain of their offspring. It is evident from a comparison of the corresponding extremes of black pigmentation in the tortoise offspring that more black occurs in the coat of the young from the black-and-white female, than in those from the red-and-white female. While we believe that this relation would hold generally, it should be mentioned that these two females are the only ones which have been sufficiently tested on this point.

If black-and-whites carry a large amount of black pigmentation it is to be expected that when the parents are tricolors these also will tend to show a large amount of black. On the other hand, tricolor parents of e^p red-and-whites should show a relatively small amount of black. Figure 4 illustrates this point with actual examples. It is noteworthy, furthermore, that tricolor parents do not as a rule produce both black-and-whites and e^p red-and-whites in addition to their tricolor offspring.

GOODALE and MORGAN (1913), in the previously quoted concluding paragraph of their paper, make the statement that tricolors throwing bicolor blacks (black-and-whites) "could throw some bicolor blacks which are real bicolors, not overlapped bicolors". By "real" bicolors they presumably mean black-and-whites which would breed true. According to our explanation such black-and-whites would of necessity carry the E or total-extension factor (figure 1, type 3). They cannot, however, obtain this factor from tricolor parents since tricolors themselves cannot carry E, which causes all pigmented areas to be black. Moreover, GOODALE and MORGAN present no proof that they did obtain such black-and-whites, but they believed there probably were "real bicolors" because it seemed to them that the large percentage of black-and-white offspring obtained in some of their tricolor matings could not be accounted for by the chance complete overlapping of all red areas by black. Their result might be sufficiently explained, however, by the fact already shown, that a large amount of black pigmentation and at the same time a large amount of white spotting are favorable for the production of black-and-whites. Furthermore some of the animals designated as bicolor blacks by GOODALE and MORGAN, showed small amounts of red and accordingly should be classified as tricolors.

CASTLE (1912) makes mention of races of red-and-whites and of black-and-whites which breed true, but he does not state how they can be produced. We have already shown (p. 300) that when $e^p e$ tricolors are mated together some ee red-and-whites are produced and these breed

true. Red-and-whites of this composition can also be produced in another manner. When self reds are mated to $e^b e$ tricolors, $e^b e$ black-and-whites, $e^b e$ red-and-whites, (i.e., animals of the tricolor series), or to *ee* red-and-whites, there are always some self reds produced. These self red animals must be *Ss* because one of their parents was white-spotted, and they are also *ee* since otherwise some black spotting would be visible. When they are inbred, therefore, some white-spotted offspring will result. These will breed true for the lack of black spotting, and, as a general rule, when the white spots are large, for the presence of white spotting.[10] A considerable number of red-and-whites that breed true have been obtained in this manner.

To produce black-and-whites which breed true a somewhat similar procedure is followed. In this case we want to introduce the *E* factor, which causes the black to be extended throughout the pigmented part of the coat. This is done by crossing a self black (*EESS*, figure 1, type 1) with an *ee* red-and-white. The F_1's will all be self black. Their composition will be *EeSs*. When they are inbred we should expect one out of sixteen of the offspring to have the composition *EEss* (figure 1, type 3). These would be the black-and-whites which breed true. We have obtained animals of this kind by following the above method. One of them, ♂ 356.3, when mated to other black-and-whites had 20 offspring, all black-and-whites. When mated to *ee* red-and-whites, he had 18 offspring, all of which again were black-and-whites. We have neglected to mate *EE* black-and-whites to self reds, but we have made a mating using an *Ee* black-and-white. The resulting offspring consisted of 9 self blacks and 8 self reds. *EE* black-and-whites mated to self reds should get all self black offspring.

From the foregoing at least two methods can be derived whereby e^b black-and-whites can be distinguished from black-and-whites carrying the *E* factor. Inspection of the third type of mating in table 4 will show that when $e^b e^b$ black-and-whites are mated to *ee* red-and-whites tricolors tend to make up a majority of the offspring. In a similar kind of mating when *EE* black-and-whites are used, all of the offspring are black-and-whites. The above is the first method. The second method, which requires fewer offspring to determine the point at issue, consists in mating the black-and-whites to be tested to self reds. Animals homozygous for e^b produce only tortoises, while those homozygous for *E* produce only self blacks. It must be borne in mind, however, that we may

[10] In a few cases we have obtained selfs when two white-spotted animals of this sort were bred together.

have animals of the formula Ee^p. These when bred to self reds should produce approximately equal numbers of self blacks and tortoises.[11]

When EE black-and-whites are crossed with e^pe^p animals of the tricolor series, the resulting offspring should be Ee^p black-and-whites. When inbred these should get the following three kinds of offspring: 1 EE: 2 Ee^p: 1 e^pe^p. The first kind would be black-and-whites which breed true; the second kind would be black-and-whites like the F_1 parents; and the third kind would be animals of the tricolor series, i.e., black-and-whites, red-and-whites or tricolors. This is the sort of mixture GOODALE and MORGAN thought they obtained from tricolor × tricolor matings. While we have not made the above cross, other work now in progress has demonstrated beyond a doubt that E, e^p and e form an allelomorphic series, which would give a result as indicated.

It may be of interest in this connection to discuss a cross made by GOODALE and MORGAN (1913, p. 332). A tricolor female when mated to a self black male produced one self black, one self red and a tortoise. She had previously been mated to a self red and had produced 7 self red offspring. Her composition would therefore be e^pe. There is nothing except the present mating by which to determine the factorial composition of the self black male. GOODALE and MORGAN conclude:

> "This male appears to have been homozygous as regards lack of spotted white [SS], heterozygous for [self] black (Bb) [Ee], and also heterozygous for some factor that causes black to appear in spots [e^pe], i.e., a factor analogous to the factor commonly recognized as the white spotting factor."

According to this interpretation (leaving S out of consideration) the self black male would carry our three factors, E, e^p and e. This is untenable by our hypothesis, since an individual can carry only two of the factors making up an allelomorphic series. Our interpretation is that the self black in question was Ee. We have already shown that the tricolor female is e^pe. $Ee \times e^pe = 1\ Ee^p : 1\ Ee : 1\ e^pe : 1\ ee$. This means that from the above kind of mating the expectation is 2 self blacks: 1 tortoise : 1 self red. The actual result, 1 self black: 1 tortoise: 1 self red, is quite close to expectation. Further proof of the correctness of this interpretation will be given in the forthcoming paper already referred to.

Although CASTLE (1912) described all the characters involved in the tricolor series of guinea-pigs and how one character might mask another, he made no attempt to enumerate the factors by which these char-

[11] We have a fairly large amount of data on hand confirming this, but it is being reserved for a later paper.

acters are represented in the germ-cell. Neither did he attempt to explain the relation of the factors to each other. This has been attempted by LITTLE (1913, p. 42). The latter uses the symbol *T* to represent the self black condition. This corresponds to our *E* factor. The tortoise condition is represented by *t*, which corresponds to our c^p factor. In addition he postulates the factor *E*.

"*E* = *extended black pigmentation necessary for the production of any* black hairs on the coat, and its allelomorph *e* = the loss of black pigment from the coat, producing yellow animals.

"Thus, if a race of tortoise animals existed, formula *EEtt*, we should have a homozygous race of tortoise animals (which in fact exist). If, however, the tortoise animals were of the formula *Eett* we should expect young of three kinds:

(a) *EEtt* tortoise, (b) *Eett* tortoise, (c) *eett* yellow. Types (a) and (b) having *E* present would be able to form black pigment on the coat; but type (c), lacking *E*, would be *yellow* [red] in appearance, though it would be *potentially tortoise*."[12]

If animals of the formula *cctt* are self reds that are potential tortoises, then animals of the formula *ccTT* must be *self reds that are potentially self blacks*. Therefore, according to his interpretation, all self red animals are potentially either self blacks or tortoises. This leads to some difficulties. Suppose that a self red of the formula *ccTT* (and hence potentially a self black) were crossed with a tortoise, *EEtt*, all the offspring should be *EcTt*, or self blacks. We have crossed many self reds, extracted from self black matings, and which, therefore, according to LITTLE's hypothesis should carry the *T* factor, with tortoises, and have never obtained any self blacks.

If LITTLE's hypothesis is correct, heterozygous self black animals of the formula *EcTt* bred to self reds of the formula *cctt* or *ccTt* should produce three kinds of offspring: self blacks, tortoises and self reds. We have mated a fairly large number of heterozygous self blacks, obtained in various ways, to self reds extracted from various kinds of matings and have always obtained either approximately half self blacks and half tortoises or half self blacks and half self reds, but never all three kinds of offspring from any one mating.

All the evidence we have at hand seems to indicate, therefore, that self reds are potentially neither self blacks nor tortoises, but simply what they appear to be, self reds. Such being the case, we feel justified in asserting that the relationship of self black, tortoise and self red cannot be adequately explained on the basis of two independent sets of allelomorphs.

[12] Original not italicized.

SUMMARY

1. The factors more or less directly concerned with tricolor inheritance in guinea-pigs are described and their inter-relations shown by means of table 1 and figure 1.

2. In the experimental results it is shown: (1) that tricolors may be of two kinds—those homozygous and those heterozygous for the e^p or black-spotting factor; (2) that black-and-whites of tricolor parentage are also either homozygous or heterozygous for the same factor; and (3) that red-and-whites of tricolor parentage may carry the e^p factor in a homozygous or heterozygous condition, or it may be altogether absent, in which event the red-and-white breeds true.

3. Red-and-whites may be tested for the presence of the black spotting factor by mating them to homozygous self reds. If the factor is present there will be tortoises among the offspring; if it is absent they will be all self reds.

4. Black-and-whites of the tricolor series, homozygous for e^p, produce only tortoises when mated to homozygous self reds; if they are e^pe, half of the offspring are tortoises and the other half self reds. Both these kinds of black-and-whites, therefore, produce *some* tortoises, but *no* self blacks when mated to self reds.

5. Black-and-whites carrying *E* may be of three kinds, (1) *EE*, (2) Ee^p and (3) *Ee*. The first kind when mated to homozygous self reds produce all self blacks; the second kind, half self blacks and half tortoises; the third kind, half self blacks and half self reds. Black-and-whites carrying *E*, therefore, *always* produce *some* self blacks when mated to self reds, but in some instances (when Ee^p) also produce tortoises.

6. *EE* and *Ee* black-and-whites were produced by mating self blacks to *ee* red-and-whites and inbreeding the F_1 self blacks. Some of the F_2 generation are black-and-whites which are either *EE* or *Ee*. The first kind when mated together breed true.

7. Animals of the tricolor series carrying a large amount of black pigmentation (e^p) and also a large amount of white spotting tend to produce a comparatively large number of black-and-whites and no e^p red-and-whites. Those carrying a small amount of black pigmentation tend to produce a comparatively large number of e^p red-and-whites and no black-and-whites.

8. The statement is made, but complete experimental proof is reserved for a later paper, that the three factors, *E*, complete extension

of black pigment, e^p, partial extension, and e, non-extension, form an allelomorphic series.

LITERATURE CITED

CASTLE, W. E., 1905 Heredity of coat characters in guinea-pigs and rabbits. Carnegie Institution of Washington, Publication No. 23, 78 pp., 6 pls.

1909 Studies of inheritance in rabbits. Carnegie Institution of Washington, Publication No. 114, 68 pp., 4 pls.

1912 On the inheritance of tricolor coat in guinea-pigs, and its relation to Galton's law of ancestral heredity. Amer. Nat. **46**: 437-440.

1914 Some new varieties of rats and guinea-pigs and their relation to problems of color inheritance. Amer. Nat. **48**: 65-73.

COLE, L. J., 1914 Studies on inheritance in pigeons: 1. Hereditary relations of the principal colors. Rhode Island Exp. Station, Bulletin No. 158, pp. 311-380, pls. 1-4.

GALTON, F., 1889 Natural inheritance. 254 pp. London and New York: Macmillan Co.

1897 The average contribution of each several ancestor to the total heritage of the offspring. Proc. Roy. Soc. London **61**: 401-413.

GOODALE, H. D., and MORGAN, T. H., 1913 Heredity of tricolor in guinea-pigs. Amer. Nat. **47**: 321-347.

LITTLE, C. C., 1913 Experimental studies of the inheritance of color in mice. Carnegie Institution of Washington, Publication No. 179, pp. 11-102, pls. 1-5.

WHITING, P. W., 1915 The tortoiseshell cat. Amer. Nat. **49**: 518-520.